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Full Length Research Paper

Alternative products for overcoming dormancy in grapevines 'Isabel Precoce'

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The stage of dormancy is an important physiological condition in the behavior of temperate climate fruit trees, and it is reached at the end of the vegetative cycle, when the leaves are fallen. In general, a cold period is needed to overcome the state of dormancy and promote uniform buds sprouting, being therefore a limiting factor for grape production in Brazil. There are chemicals used in breaking dormancy of the vines, allowing the production. However, there is need to restrict the use of those products in the management of orchards, defending sustainable fruit production systems. For this purpose, alternative products, such as garlic extract, have shown promising results in overcoming dormancy in fruit trees. The objective of this study was to evaluate the effect of different doses of natural garlic extract (NGE) (*Allium Sativum* L.) and Bioalho® (Natural Rural S.A.) added or not to mineral oil (MO) (2%), in overcoming dormancy of grapevines buds of the cultivar 'Isabel Precoce' (*Vitis Labrusca L.*), soil and climate conditions of the region of Lavras – Minas Gerais. The results of this study showed that the action of NGE and Bioalho® in breaking dormancy in different intensities, and the treatments with NGE 15% + MO (2%), NGE 20% with and without MO (2%), Bioalho® 15% with and without MO (2%) and Bioalho® 20% with and without MO (2%) had the best results in the induction of the shoots of the vines 'Isabel Precoce'.

Key words: Garlic extract, buds, sprouting, grapes.

INTRODUCTION

The fruit trees of temperate climate have a mechanism of protection that allows their survival in adverse weather conditions, known as dormancy (Campoy et al., 2011). According to Perez and Lira (2005), temperature is the most important environmental variable in the induction processes and overcoming of dormancy of these fruit trees. Low temperatures lead these plants to a state of physiological inactivity. A period with accumulation of hours of chilling is necessary to overcome this stage of dormancy and start a new cycle of vegetation, without delay and within uniformity to the shoots. In the case of vines, a leading fruit of temperate climate, this period can vary from 50 to 400 h, with temperature at 7°C, depending on the cultivar (Botelho and Muller, 2007).

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In this sense, Hawerroth et al. (2013) claimed that the dormancy is one of the main factors that influence the production of temperate fruit trees. Hence insufficient cold periods cause irregularities on the sprouting and the development of these plants when grown in hot climates (Bonhomme et al., 2005), as found in Brazil. Therefore, the use of chemicals to overcome dormancy is key in order to improve produce in these regions (Botelho and Muller, 2007).

Currently, the hydrogenated cyanamide (H_2CN_2) has been the most used compound to induce sprouting of temperate fruit trees (Hawerroth et al., 2013), mainly in vines (Botelho and Muller, 2007). However, the H_2CN_2 is a product considered as toxic, since it has been classified by the Environmental Protection Agency of the United States in the category of highest toxicity (Category I) (Settimi et al., 2005).

Given the aforementioned, there is the need of alternative substances with similar effect of the H_2CN_2, to be used as dormancy handlers and thus make the production in hot climates more sustainable. Botelho et al. (2010) included the garlic extract as an alternative substance to be employed, which provided good results.

The aim of this study was to evaluate the effect of different doses of natural garlic extract (EAN) and Bioalho® added or not to mineral oil (OM), in overcoming the dormancy of buds of the cultivar 'Isabel Precoce' in the edaphoclimatic conditions of the region of Lavras - MG.

MATERIALS AND METHODS

The experiment was conducted in the Orcharding Sector, at the Universidade Federal de Lavras (UFLA), in Lavras, Minas Gerais (21° 14' 06" S, 45° 00' 00" W, and altitude 918 m). The climate of the region according to the classification of Köeppen, modified by Vianello and Alves (1991) is of type Cwb, mild temperate (mesothermal), being the average annual temperature and precipitation of 19.4°C and 1,529 mm, respectively. According to Alvarenga et al. (2002), Lavras has an average annual number of 100 chilling units and only in years with very intense winter, that number could reach 150 h. The vegetative material used in the experiment was the cultivar Isabel Precoce, with 3-year-old grafted on rootstock 'Paulsen 1103', by the method of grafting Omega type. Vines were planted in September, 2010, being conducted in the cordon-spur, with 1.0 m spacing between plants and 3.0 m between rows. Cultural and phytosanitary treatments were performed as recommended by Chalfun et al. (2002) and fertilizing as recommended by Ribeiro et al. (1999).

Fruiting vines pruning was performed in August, 2014, leaving 4 to 6 buds per branch. The treatments were applied on the same day, through direct brush stroke on the dormant buds, until complete wetting. Thirty days after application of the treatments, a count of sprouted buds per plant was held, and therefore, the assessed percentage which sprouted was obtained.

The products used in the treatments were the Bioalho® (extrato de alho, Natural Rural S.A., Araraquara, Brasil) - commercial product obtained by cold extraction of garlic extract through pressing, being totally soluble in water and added adjuvants; hydrogenated cyanamide (H_2CN_2) (Dormex®, 520 g L^{-1} H_2CN_2, Basf S.A, Guaratinguetá, São Paulo) - growth regulator of the group of the carbamidas; mineral oil (OM) (Assist®, 750 ml L^{-1} mineral oil, Basf S.A., Guaratinguetá, São Paulo) - mixture of paraffinic hydrocarbons, paraffinic and aromatic cycle from saturated and unsaturated petroleum distillation; and natural garlic extract (NGE) – homemade product made with the cultivar 'Chonan Roxo' of the species *Allium Sativum* L. For its obtainment, the shell was removed followed by the subsequent grinding of the cloves in a domestic blender (Wallita®). After the grinding, the dough was simultaneously pressed and filtered with a household potato masher and a cotton cloth, respectively (Figure 1).

The following treatments were applied: 1) Control (water); 2) NGE 5%; 3) NGE 10%; 4) NGE 15%; 5) NGE 20%; 6) NGE 5% + MO 2%; 7) NGE 10% + MO 2%; 8) NGE 15% + MO 2%; 9) NGE 20% + MO 2%; 10) Bioalho® 5%; 11) Bioalho® 10%; 12) Bioalho® 15%; 13) Bioalho® 20%; 14) Bioalho® 5% + MO 2%; 15) Bioalho® 10% + MO 2%; 16) Bioalho® 15% + MO 2%; 17) Bioalho® 20% + MO 2%; 18) H_2CN_2 2% and 19) MO 2%. The experimental design was completely randomized, with three replications and experimental plot consisting of a plant. Data were analyzed through analysis of variance (ANOVA) on the statistical program SISVAR version 5.3 (Build 77). Significant comparisons were generated using the Scott-knott test at 5%, and regression analysis.

RESULTS AND DISCUSSION

The percentage of breaking buds of the cultivar 'Isabel Precoce' was influenced by the application of dormancy agents in the plants, since the result of analysis of variance was significant. In addition, the effect of the dormancy handlers, in other words, sprouted buds 30 days after the product application varied according to doses applied. Figure 2 presents the average sprouting in relation to each treatment, similar to a correlation between product and percentage of sprouted buds.

Vines which received the treatments NGE 20%; Bioalho® 15%; NGE 15% + MO 2%; NGE 20% + MO 2%; Bioalho® 20% + MO 2%; Bioalho® 20% and Bioalho® 15% + MO 2% showed, respectively 87, 90, 93, 95, 96, 96 and 97% of sprouted buds, 30 days after their application. These were the treatments which provided greater efficiency in overcoming dormancy of buds in vines, not statistically different from the conventional treatment with H_2CN_2 (Dormex®), which obtained 100% of buds sprouted.

The treatments with NGE 5%; NGE 10%; NGE 5% + MO 2%; NGE 15%; NGE 10% + MO 2%; MO 2%; Bioalho® 10% + MO 2%; Bioalho® 10%; Bioalho® 5% and Bioalho® 5% + OM 2% showed inferior results to the treatments described previously, but were better than the witness. In that way, some budding improvement occurs with doses less than 15%. Botelho et al. (2009) studied the effect of the application of garlic extract (3%) with and without vegetable oil (1%) on the induction of sprouting on the vine 'Isabel Precoce'. The only treatment that stimulated the sprouting was garlic extract (3%) added to mineral oil, however it was partial and therefore inefficient.

Oliveira et al. (2009) observed that garlic extract had influence on overcoming the dormancy of buds in vines, and the treatment that showed similar results related to

Figure 1. Obtaining the Natural Garlic Extract (NGE) with removal of the bark of garlic loves (A); grinding of garlic cloves; (B); pressing and filtering of the dough (C and D) and extraction of NGE (E and F).

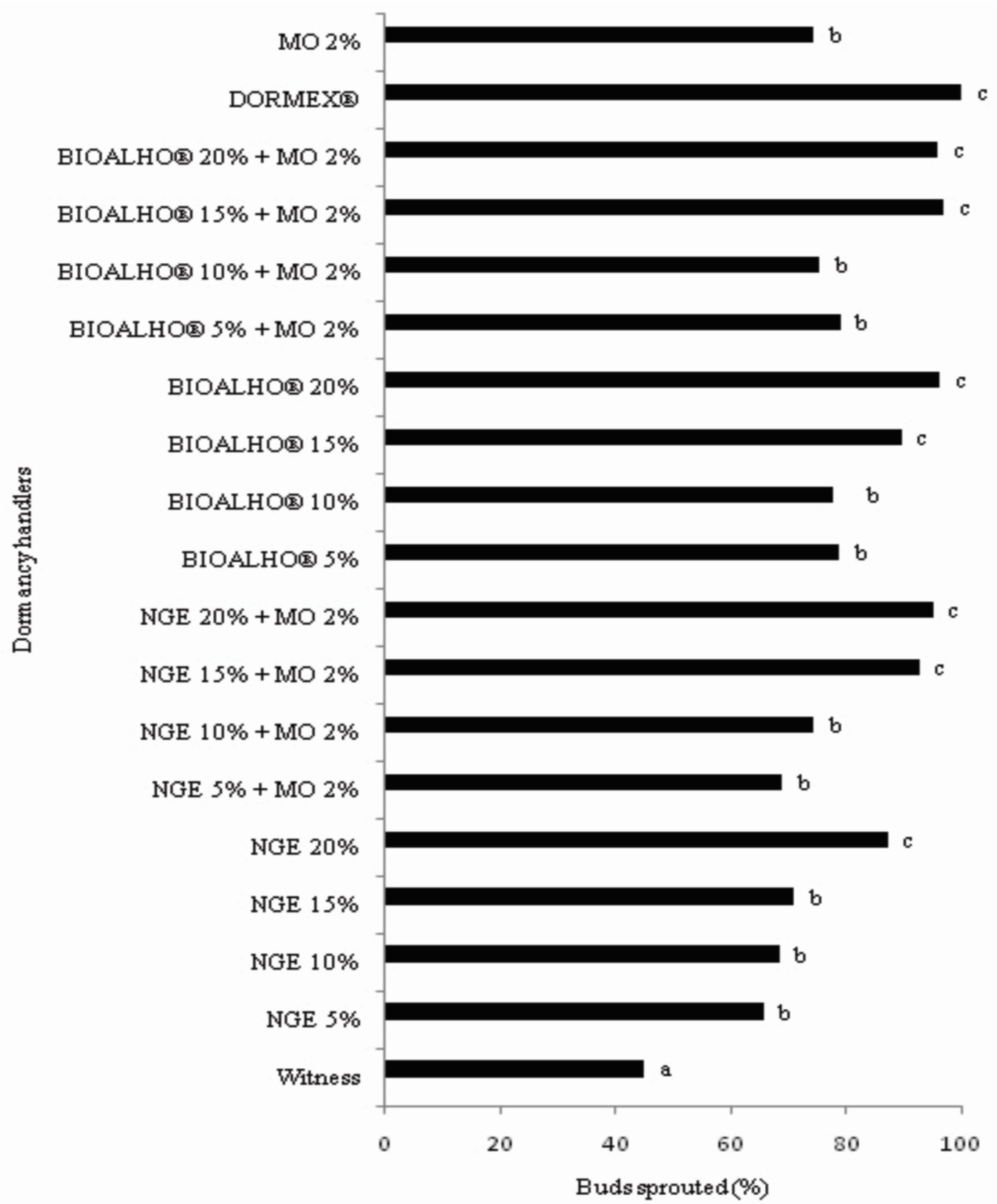

Figure 2. Percentage of sprouted buds of the cultivar 'Isabel Precoce' 30 days after application of the dormancy handlers, Lavras, UFLA, MG, 2013. Means with same letter do not differ, by the Skott-knott test ($p < 0.05$).

addition of H_2CN_2 over the anticipation of sprouting was the garlic extract (5%) added to mineral oil (4%). Rady and Seif El-Yazal (2014) identified in their study that the use of garlic extract in order to anticipate sprouted buds increased the yield in apple trees. In addition to these authors, Kubota et al. (2000) and Botelho et al. (2010) also found that garlic extract had a great potential in breaking dormancy in grapevines.

Generally speaking, it turns out that the alternative products EAN (homemade product) and Bioalho® (commercial product) proved to be effective in overcoming dormancy and sprouting induction in 'Isabel Precoce' cutivar. The doses tested in this work that had greater effect on overcoming dormancy for this cultivar

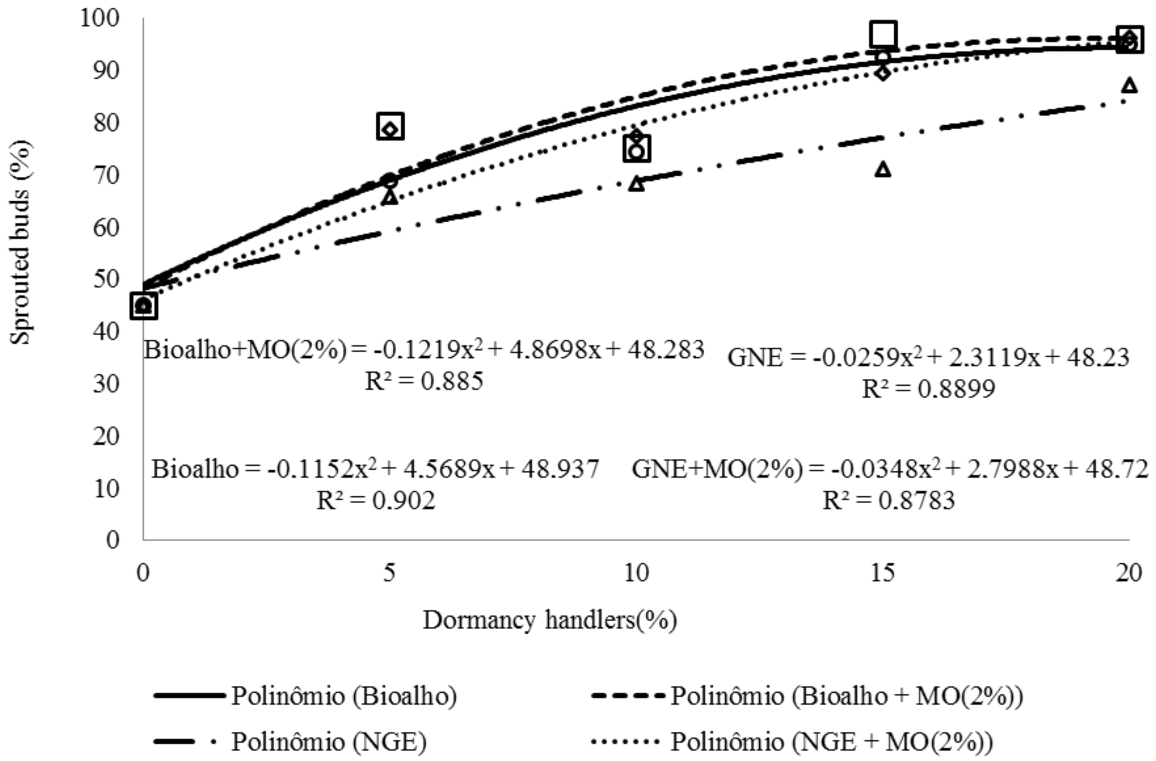

Figure 3. Sprouted buds (%) of the cultivar Isabel Precoce 30 days after the application of different doses of dormancy handlers – NGE, NGE + MO (2%), Bioalho® and Bioalho® + MO (2%), UFLA, Lavras, MG, 2013.

were the doses greater than or equal to 15% for both products (EAN and Bioalho®). However, in the case of EAN at the dosage of 15%, the addition of mineral oil provided better result. Thus, it is observed that the MO acts as a booster to the NGE effect, since the doses of NGE added to MO showed better results when compared to the doses of NGE without MO treatment. In Figure 3, the percentage of sprouted buds of the cultivar 'Isabel Precoce' as a function of the application of increasing doses of NGE and Bioalho® is shown. The results showed that the increase in doses of both NGE and Bioalho® promotes increase in percentage of sprouted buds of the cultivar 'Isabel Precoce', 30 days after application of the treatments. It turns out that there is an optimal concentration of both the homemade product NGE and the commercial product Bioalho® which provides maximum budding, and concentrations greater than this may result in a reduction in the percentage of sprouted buds. The doses of Bioalho®, Bioalho® + MO 2%, NGE and NGE + MO 2% are 20, 20, 45 and 40%, respectively, which generate the maximum budding in the cultivar 'Isabel Precoce'. This means that concentrations higher than these can cause phytotoxicity to the plant and consequently reduce the percentage of sprouted buds. In this way, the NGE, homemade product, characterized by extraction and production, with low toxicity and high efficiency in shoots stimulation, is a potential substitute for H_2CN_2 in overcoming dormancy of the cultivar 'Isabel Precoce'. Similarly, the commercial product Bioalho® can also substitute the H_2CN_2. However, it should be remembered that there is an optimal dose that promotes break in dormancy, and higher doses can cause antagonistic effects, thereby reducing the shoots.

Conclusion

The results of this study demonstrate that there is a positive action in NGE and Bioalho® on overcoming the dormancy for 'Isabel Precoce' plants. The treatments NGE 15% added to MO 2%, NGE 20% added or not to MO 2% and Bioalho® 15 and 20 added or not to MO 2%, stimulated the budding of the vines 'Isabel Precoce', which showed similar effects as the conventional treatment with H_2CN_2.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Symbiotic nitrogen fixation and nitrogen budget of Brazilian soybean [*Glycine max* (L.) Merril] varieties introduced in Benin using ^{15}N isotopic dilution method

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Nitrogen-15 isotopic dilution method was used to estimate biological nitrogen fixation (BNF) and nitrogen (N) budget of fourteen (14) soybean varieties using maize as reference crop. The experiment was carried out at Sekou in Southern Benin. The amount of N derived from air (Ndfa kg N ha^{-1}) ranged from 51 for variety BRS 261 to 148 for variety Canarana. In a scenario where the soybean shoot dry matter and grains were removed from the field after harvest and only the fallen leaves were incorporated into the soil, the N budget ranged between -91 (Canarana) and -17 kg N ha^{-1} (BRS 260). When only soybean grain was exported from the fields and fallen leaves and shoot dry matter are incorporated into the soil, the N budget varied from 7 (BRS 261) to 74 kg N ha^{-1} (BRS Garantia). The study showed that Canarana, TGX 1448 2E and BRS Paraiso soybean varieties fixed the highest amount of N among the 14 varieties. The inclusion of those soybean varieties in cereal-based cropping systems would help reduce N inputs and improve soil and crop productivity in farming systems in Benin.

Key words: ^{15}N enrichment technique, N_2 fixation, N balance, soil fertility, Benin.

INTRODUCTION

In tropical regions, nitrogen (N) deficiency is frequently one of the major factors limiting crop yields and N fertilization is increasingly applied to increase the crop yields (Reinhold-Hurek and Hurek, 2003). Managing nitrogen inputs in crop production system to achieve economic and environmental sustainability is a major challenge facing agriculture (Haque and Sattar, 2010). In this context, biological fixation (BNF) has become one of the most attractive strategies for the development of sustainable agricultural systems (Hayat et al., 2008). However, farmers in West Africa are often reticent to adopt legume cover crops such as *Mucuna* species that

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are not used for human consumption or without a direct economic profit, in spite of the positive impact on restoring soil fertility (Mayong et al., 1999). Several authors proved interests to use dual-purpose grain legumes in farming systems (Sanginga et al., 2003; Houngnandan et al., 2009). The integration of grain legumes such as dual-purpose soybean [*Glycine max* (L.) Merril] into rice or maize-based systems has been reported to greatly enhance rice and maize productivity and the sustainability of the production systems in the West African savannas (Houngnandan et al., 2009). Indeed, soybean is the most important cropped legume in the world due to its high protein content, its lower susceptibility to pests and diseases, better grain storage quality, huge leaf biomass yield, high ability to fix nitrogen in association with *Bradyrhizobium* in the root nodules and it N contribution to subsequent crops (Mafongoya et al., 2009). Since, interest in growing soybean has increased in Benin, the exploitation of soybean could be an attractive strategy for sustainable agricultural production in highly degraded soils in the country. But to obtain such a beneficial residual effect after legumes compared to non-legumes, it is expected that the amount of fixed N returned by the legumes to the soil must be greater than the amount of soil N in the harvested grain (Sanginga et al., 2002; Schipanski et al., 2010). It is widely recognized that soybean crops often require more N (or export more N in grain) than they fix and this often results in a net negative contribution to the N balance in the cropping systems (Jaynes and Karlen, 2008; Salvagiotti et al., 2008). The role of BNF, especially in legumes, is well established and documented but it has been reported that various varieties or cultivars of grain legumes show significant differences regarding their ability to support BNF (Hayat et al., 2008; Singh and Shivakumar, 2010).

It has been reported that in various cultivars of grain legume, the estimation of N_2 fixation depends largely on the methods used (Hardarson et al., 1993). A number of methods have been developed to quantify biological nitrogen fixation, but each method has its own merits and limitations (Herridge et al., 2008; Unkovich et al., 2008). The choice of a particular method depends on the type and site of the experiment, the available resources and the species and system in question (Gathumbi et al., 2002; Schweiger et al., 2014). The most commonly methods used to determine N fixation in legumes are: N difference, ureides, acetylene reduction assay, ^{15}N natural abundance and ^{15}N enrichment methods (Forrester et al., 2006; People et al., 2009). Among these methods, ^{15}N enrichment and ^{15}N natural abundance are considered to be time integrated methods to determine N_2 fixation under natural conditions (Boddey et al., 2000; Unkovich and Pate, 2000). But it is generally believed that isotope dilution utilizing enriched ^{15}N gives the most accurate quantification of nitrogen turnover in the main processes of the nitrogen cycle (Chalk, 1985). This study aims to estimate the biological N_2 fixation of fourteen Brazilian soybean varieties introduced in the Southern Benin by the ^{15}N isotopic dilution method and also estimate the total net N inputs in soil system.

MATERIALS AND METHODS

The study was carried out at the Application and Production Farm of the Faculty of Agronomic Sciences of the University of Abomey-Calavi located at Sekou (2°14' - 2°26 E and 6°37' - 6°40' N) in Benin from July to October, 2009. The climate is warm and subequatorial humid with a bimodal rainfall distribution. According to the weather station ASECNA (from year 1988 to year 2008), the annual mean temperature was between 26 and 29°C and the annual mean rainfall was between 1000 and 1400 mm. However, during the experiment, mean precipitation and temperature near the site were 708 mm and 27°C, respectively. The experimental farm has been established on a typical "*terre de barre*" soil, classified by Food and Agriculture Organization-United Nations Educational, Scientific and Cultural Organisation as Rhodic Ferralsol. Rhodic Ferralsol is a highly degraded soil due to high demographic pressure; intensive cropping with less or no financial capacity for farmers to apply chemical fertilizers, while fallow in the "*Terre de Barre*" area disappeared. The experimental farm soil had a sandy-clay texture, and its main chemical characteristics are presented in Table 1.

Fourteen soybean varieties were introduced from three different countries.

(1) Ten Brazilian varieties: BRS 260, BRS 261, BRS 262, BRS 268, BRS Ipameri, BRS Santacruz, BRS Luziania, BRS Paraiso, BRS Garantia, BRS Vencedora;
(2) Two varieties introduced from Ghana (Anidazo and Jenguma) and one from Côte d'Ivoire (Canarana). They were all originally from Brazil.
(3) One variety of International Institute of Tropical Agriculture (IITA): TGX 1448 2E which was used as control because it was already in the extension systems few years ago in Benin.

The microbiological material was the inoculum prepared from IRAT FA3 strain of specific *Bradyrhizobial* bacteria fixed on the peat. IRAT FA3 strain was selected by the Nitrogen Fixation in Tropical Agricultural Legumes/Microbial Resource Centre Rhizobium project and used to inoculate strictly nodulating soybean varieties adapted to tropical agricultural zones in Africa and Latino America (Houngnandan et al., 2008).

Experimental design

The field experiments were a randomized completed block design with three replicates or blocks. Each block contained fourteen plots. Each plot contained four planting lines. The space between rows was 50 cm and the inter-plant spacing was 20 cm. On the planting row, soybean was seeded at a 5 cm within-row space. Four seeds were sown per hole and later thinned to three 14 days after. Soybean seeds were first inoculated with IRAT FA3 strain of *B. japonicum* containing an approximate density of 10^8 viable rhizobia per seed before sowing. One maize plot was sown aside each soybean main plot and used as a reference plant for soybeans varieties. The space between rows was 75 cm and the inter-plant spacing was 40 cm. The isotopic dilution method was applied using micro plots installed in all plots (1 m width × 1 m length). The micro plots of soybean and the reference micro plots were enriched with 20 kg N/ha of 5.73% ^{15}N atom excess labeled urea dissolved in

Table 1. Physico-chemical properties of soil.

Depth	pH (H_2O)	N (%)	C (%)	OM (%)	Total P (ppm)	Available P (ppm)	Exchangeable cations (meq/100 g)			CEC (meq/100 g)	Sand (%)	Silt (%)	Clay (%)
							Mg^{2+}	Ca^{2+}	K^+				
0-20	5.7	0.08	0.84	1.44	419.77	5.84	1.33	2.5	0.25	12	79.5	2.75	17.75

N: Total nitrogen; C: carbon; P: phosphorus; CEC: cation exchange capacity.

water when the remaining plots received 20 kg N/ha of unlabelled urea solution. All plots received 100 kg ha^{-1} of P_2O_5 as triple superphosphate.

Plants sampling

Soybean and maize shoots were collected at the first sampling (at the flowering time 10 weeks after planting). In each plot, eight plants and two plants were randomly sampled, respectively for soybeans and maize. Shoots and roots of soybeans and maize were separated. Roots were washed to remove adhering soil. Nodules were removed and counted and dry weight was taken. Soybean shoots and roots and the reference plants were dried at 65°C for 72 h and ground. Nitrogen content of both plant (soybean and maize) was analyzed by the Kjeldahl method. The labeled soybean and maize sampled were ball milled, well packed and sent to the Seiberdorf laboratory (International Atomic Energy Agency) for ^{15}N atom% excess analysis using an isotope ratio mass spectrometer.

At the second sampling (at the harvest), straws and grains were sampled on an area of 6 m² (3 m × 2 m) per plot. Total fresh weights of straws and grains harvested were taken. Then, a sub-sample of straw and grain was taken for yield calculation. Total N straw and grain was analyzed by the Kjeldahl method.

METHODOLOGY AND CALCULATIONS

Calculation of % Nitrogen derived from atmosphere (Ndfa) using isotope dilution method

Legume and non N_2-fixing reference plants were grown in soil receiving the same amount of ^{15}N-labelled N fertilizer. The dilution of the ^{15}N taken up from the residual enriched fertilizer by ^{14}N derived from the atmosphere relative to that of non-fixing reference plants was used to calculate the percentage of N derived from atmosphere (%Ndfa) by the legume using Equation 1 (Fried and Middelboe, 1977; Unkovich et al., 2008):

%Ndfa = 1 - (Atom% excess of soybean) / (Atom% excess of maize) × 100 (1)

The amount of N symbiotically fixed by soybean (BNF, kg.ha^{-1}) was calculated by multiplying the total N in soybean plant samples by the %Ndfa.

Calculation of nitrogen balance (N balance)

The nitrogen balance at the soil surface is the difference between the total quantity of nitrogen inputs to the soil surface and the quantity of outputs that are released from the soil, annually (Vassiliki et al., 2012). The total quantity of nitrogen inputs to the soil during the process of agricultural production are those coming from mineral fertilizers and organic manure applied to agricultural land, the fixation by leguminous crops and the wet and dry deposition from the atmosphere. The outputs (removals) of N are defined as the N content of crops removed from the field by harvest or by grazing (Vassiliki et al., 2012). In this study, the inputs were fertilizer and non-fertilizer (biological nitrogen fixation) and the outputs were nutrients removed in harvested products (Adu-Gyamfi et al., 2007). Nutrient losses out of the systems (leaching, erosion, overland and lateral transport) were not included. In the calculation, two scenarios were examined. In the first scenario (budget 1), it is assumed that the soybean shoot dry matter and grains are removed from the field after harvest and only the fallen leaves are incorporated into the soil. In the second scenario (budget 2), it is assumed that only soybean grains are exported from the fields and the roots, while fallen leaves and shoot dry matter are incorporated into the soil. The corresponding equations were:

Budget 1 = (Amount of N applied + Amount of N fixed) – (Amount of N in shoot dry matter + Amount of N in grains) (2)

Budget 2 = (Amount of N applied + Amount of N fixed + Amount of N in shoot dry matter) - Amount of N in grains (3)

Calculation of dry matter yield and grain yield at harvest

Yield (kg DM ha^{-1}) = Dry matter factor × (total fresh weight × 10 000) / Effective area (IAEA, 1990) (4)

The dry matter factor is the quotient between dry sample weight and fresh sample weight.

Statistical analysis

Statistical analyses were carried out using SAS software version 9.2. One-way analysis of variance (ANOVA) was performed to determine the statistical differences among the soybean varieties. When significant differences ($p < 0.05$) were noticed, a Student-Newman-Keuls test was used to compare the means.

Table 2. Nodulation and biomass production of soybean grown at Sekou station in 2009.

Variety	Nodule number (number plant^{-1})	Nodule dry weight (mg plant^{-1})	Shoot dry weight at flowering (g plant^{-1})	Dry matter Yield at Harvest (kg ha^{-1})
Anidazo	34^{ab}	283.6^{ab}	9.4^{bcd}	2525^{bcd}
BRS 260	33^{ab}	412.6^{a}	4.3^{d}	1015^{dc}
BRS 261	47^{ab}	254.5^{ab}	4.8^{cd}	1135^{dc}
BRS 262	40^{ab}	317.2^{ab}	5.2^{cd}	1260^{dc}
BRS 268	29^{b}	179.0^{b}	5.2^{cd}	1257^{dc}
BRS Garantia	48^{ab}	380.7^{ab}	12.5^{ab}	3454^{ab}
BRS Ipameri	40^{ab}	284.5^{ab}	10.2^{abcd}	2755^{abcd}
BRS Luziania	46^{ab}	345.0^{ab}	8.5^{bcd}	2235^{bcd}
BRS Paraiso	44^{ab}	359.1^{ab}	11.4^{abc}	3132^{ab}
BRS Santacrutz	36^{ab}	293.8^{ab}	5.2^{cd}	1266^{dc}
BRS Vencedora	60^{a}	353.9^{ab}	6.2^{bcd}	1545^{bcd}
Canarana	36^{ab}	131.7^{b}	15.9^{a}	4065^{a}
Jenguma	39^{ab}	184.8^{b}	12.3^{ab}	3396^{ab}
TGX 1448 2E	35^{ab}	187.0^{b}	12.1^{ab}	3336^{abc}
Min	24	69.1	2.8	958
Max	74	500.0	18.2	4157
Mean	41	283.4	8.8	2313

Means followed by a same letter in the same column are not significantly different at $p < 0.05$ according to Student Newman-Keuls test.

RESULTS

Nodulation and biomass production

Nodulation was observed on all soybean cultivars (Table 2) and differences among cultivars in nodule number and nodule dry weight were significant (p <0.05). The lowest nodule number (29) was recorded by BRS 268, but Canarana had the lowest nodule dry weight (131.7 mg plant^{-1}). The highest nodule number and nodule dry weight were observed, respectively on BRS Vencedora (60) and BRS 260 (412.6 mg plant^{-1}). But the control (TGX 1448 2E) had 35 nodules per plant which weighed 187 mg.

There were highly significant difference (P<0.001) among soybean varieties for biomass production (Table 2). At flowering and at maturity, Canarana showed the highest biomass production (15.9 mg plant^{-1} and 4065 kg ha^{-1}) and BRS 260 had the lowest (4.3 mg plant^{-1} and 1015 kg ha^{-1}). The control (variety TGX 1448 2E) produced 12.1 g of biomass per plant at flowering and 3336 kg ha^{-1} at harvest. The three best varieties in terms of biomass production were Canarana, BRS Garantia and Jenguma.

Grain yield and nitrogen accumulation

A significant difference (p<0.05) was observed on soybean varieties for grain yield and N grain yield (Table 3). The grain yield ranged between 964 (BRS 260) and 2677 kg DM ha^{-1} (Canarana). The N grain yield varied from 66 (BRS 260) to 189 kg N ha^{-1} (Canarana).

There were highly significant differences (P<0.001) among soybean varieties for total N yield (Table 3). Canarana showed the highest N yield (71 kg N ha^{-1}) and BRS 260 had the lowest (21 kg N ha^{-1}).

The control, TGX 1448 2E produced 1628 kg DM ha^{-1} grain yield. It accumulated in grains 111 and 51 kg N ha^{-1} in biomass. It was ranked third in terms of total N accumulation after Canarana and BRS Garantia at harvest and ranked fifth after Canarana, BRS Ipameri, BRS Paraiso and Jenguma among all varieties in terms of N grain yield.

Biological nitrogen fixation and nitrogen balance

All soybeans varieties derived nitrogen from atmosphere (Table 4) and differences among varieties in % of N derived from atmosphere (Ndfa) and the total N fixed (kg N ha^{-1}) were significant (p<0.001). BRS 261 had the lowest %Ndfa (42.1%) and BRS 262 had the highest (65.8%). The control TGX 1448 2E showed 59.5% Ndfa. The amount of N fixed ranged between 51 (BRS 261) and 148 kg N ha^{-1} (Canarana). Canarana, TGX 1448 2E and BRS Paraiso showed the highest amount of N fixed (Table 4).

The N balance with all shoot dry matter removed (N Budget 1) and with all shoot dry matter incorporated (N Budget 2), indicated significant differences (p<0.01) between soybeans varieties (Table 4). N budget 1 ranged

Table 3. Grain yield and nitrogen accumulation of soybean grown at Sekou station in 2009.

Variety	Grain yield (kg ha^{-1})	N grain yield (kg N ha^{-1})	N dry matter yield at harvest (kg N ha^{-1})
Anidazo	1555^{b}	105^{b}	39^{bcd}
BRS 260	964^{b}	66^{b}	21^{d}
BRS 261	1228^{b}	93^{b}	29^{cd}
BRS 262	1215^{b}	86^{b}	26^{cd}
BRS 268	1453^{b}	104^{b}	28^{cd}
BRS Garantia	1113^{b}	84^{b}	65a^{b}
BRS Ipameri	1942 ^{b}	131^{b}	42^{bcd}
BRS Luziania	1541^{b}	109^{b}	40^{bcd}
BRS Paraiso	1763^{b}	123^{b}	51^{abc}
BRS Santacrutz	1338^{b}	93^{b}	25^{cd}
BRS Vencedora	1511^{b}	102^{b}	27^{cd}
Canarana	2677^{a}	189^{a}	71^{a}
Jenguma	1750^{b}	116^{b}	48^{abcd}
TGX 1448 2E	1628^{b}	111^{b}	51^{abc}
Min	769	27	16
Max	3005	268	82
Mean	1548	108	40

Means followed by a same letter in the same column are not significantly different at $p < 0.05$ according to Student Newman-Keuls test.

Table 4. Biological nitrogen fixation and Nitrogen balance of soybean grown at Sekou station in 2009.

Variety	Ndfa (%)	Fixed N (kg N ha^{-1})	N Budget 1 (kg N ha^{-1})	N Budget 2 (kg N ha^{-1})
Anidazo	61.3^{ab}	89^{abc}	-35^{ab}	43^{ab}
BRS 260	58.7^{ab}	52^{bc}	-17^{a}	25^{b}
BRS 261	42.1^{b}	51^{bc}	-50^{ab}	7^{b}
BRS 262	65.8^{a}	74^{bc}	-18^{ab}	34^{b}
BRS 268	55.4^{ab}	73^{bc}	-39^{ab}	16^{b}
BRS Garantia	49.2^{ab}	73^{abc}	-56^{ab}	74^{a}
BRS Ipameri	46.3^{ab}	80^{abc}	-72^{ab}	11^{b}
BRS Luziania	57.3^{ab}	85^{bc}	-43^{ab}	36^{ab}
BRS Paraiso	54.7^{ab}	95^{abc}	-59^{ab}	42^{ab}
BRS Santacrutz	55.3^{ab}	65^{c}	-32^{ab}	18^{b}
BRS Vencedora	56.8^{ab}	73^{bc}	-36^{ab}	18^{b}
Canarana	57.4^{ab}	148^{a}	-91^{b}	50^{ab}
Jenguma	55.1^{ab}	89^{ab}	-53^{ab}	41^{ab}
TGX 1448 2E	59.5^{ab}	96^{ab}	-45^{ab}	56^{ab}
Min	40.2	49	-95	16
Max	56.5	143	-16	80
Mean	55,1	81	-46	34

Means followed by a same letter in the same column are not significantly different at $p < 0.05$ according to Student Newman-Keuls test

between -91 (Canarana) and -17 kg N ha^{-1} (BRS 260). N budget 2 varied from 7 (BRS 261) to 74 kg N ha^{-1} (BRS Garantia). But for the variety TGX 1448 2E grown as control, N budget 1 and N budget 2 were, respectively -45 and 56 kg N/ha.

Correlation between amount of nitrogen fixed and selected yield parameters

Correlation between amount of nitrogen fixed and some yield parameters is shown in Table 5. N fixed was

Table 5. Correlation between amount of Nitrogen fixed and some yield parameters.

Parameter	N Fixed	N grain yield	N biomass yield	N budget 1	N Budget 2	Nodule number
N fixed	-	0.940**	0.987***	-0.674*	0.872**	ns
N grain yield	0.940***	-	0.723**	-0.86***	0.571**	ns
N biomass yield	0.987***	0.723**	-	-0.585*	0.901**	ns
N budget 1	-0.674*	-0.86**	-0.585 *	-	ns	ns
N budget 2	0.872**	0.571**	0.901 **	ns	-	ns
Nodule number	ns	ns	ns	ns	ns	-

ns: No significant; *Significant (p<5%; **Significant (p<1%); ***Significant (p<1‰).

significantly and positively correlated with N grain yield, N biomass production and N budget 2, but negatively with N budget 1. No correlation was observed between N fixed and nodule number. N budget 1 and N budget 2 were, respectively negatively and positively correlated with N grain yield, N biomass production and N fixed.

DISCUSSION

Biological nitrogen fixation of the soybean varieties

These results showed that ^{15}N isotopic dilution method can be used to evaluate the percentage and amount of N derived from atmosphere by the 14 soybean varieties. The precision and accuracy of nitrogen fixation estimated with this method depend on the choice of reference plant. A prerequisite for using the method is that the legume and reference plant should not differ in the ratio of N assimilated from added ^{15}N to endogenous unlabelled N taken up from the soil (Stahl et al., 2002). The criteria for the selection of the appropriate reference crop are: having no ability to fix nitrogen, having the same ability to extract nitrogen and also the same relative nitrogen uptake profile as fixing plant (Unkovich et al., 2008). The best non-fixing reference crop usually is non-nodulating lines of the test legume (Okito et al., 2004). But when a non-nodulating line is not available, non-fixing reference mono or dicotyledonous crops (Reiter et al., 2002) or non-legume weeds (Schwenke et al., 1998) could be used. Since a non-nodulating line of soybean was not available during our study, maize was used as the reference crop. The use of maize to estimate N fixation with the ^{15}N isotopic dilution method, was previously reported (Adu-Gyamfi et al., 2007). But it may be better to choose several reference crops to estimate nitrogen fixed using ^{15}N isotopic dilution method because of variable response of the reference crops in different conditions.

In this study, percentage of Ndfa ranged between 42 and 66% and the N fixed varied from 51 to 148 kg N ha^{-1}. Similar results were obtained by several authors for inoculated soybean cultivar. For example, using the ^{15}N isotope dilution method on five promiscuous IITA soybean lines over two seasons at Mokwa, in the southern Guinea savannah of Nigeria, Sanginga (2002) reported mean value of 91 kg N ha^{-1}. In other countries, soybeans have been reported to fix 85 to 154 kg N ha^{-1} in Brazil, 26 to 57 kg N ha^{-1} in Thailand, 78 kg N ha^{-1} in Australia (Peoples and Crasswell, 1992; Salvagiotti et al., 2008).

Peoples et al. (2009) reported a mean fixation rate for North America of 144 kg N ha^{-1}. Nodulation was observed on all soybean varieties. These results confirm those of Sanginga et al., (2000) and Houngnandan et al., (2008). But the number of nodules was not correlated with amount of N fixed. There were varieties which had a lot of nodule number, but fixed a small amount of N contrary to varieties which had little nodule number with a high amount of N derived from atmosphere. For example, Canarana had 36 nodules and fixed 148 kg N ha^{-1} while BRS Vencedora having 60 nodules, fixed only 73 kg N ha^{-1}. Thuita et al., (2012) reported that increase in nodulation was not accompanied by an increase in %Ndfa. Similar results were found by De Bruin et al., (2010) who suggested that it is pertinent to know prior to the introduction of a soybean variety if inoculation can better promote nodulation and N_2 fixation.

Nitrogen balance

Nitrogen balance values were negative when shoot dry matter and grain were removed from field after harvest. These values were improved when only shoot dry matter was left in the field. Biomass production, N yield and N fixed were negatively correlated with N budget 1, but positively with N budget 2. The N export from harvested grains was for all varieties greater than the N input through symbiotic N_2 fixation, resulting in a negative N balance. This means that irrespective of the cropping system, soybean resulted in a net removal of N from the soil in spite of symbiotic N_2 fixation (Jaynes and Karlen, 2008; Salvagiotti et al., 2008). Larger amounts of N derived from air were accumulated by soybean; therefore, a large proportion of N fixed by soybean is exported from the field at grain harvest. Previous studies showed that soybean cultivation results in a net N loss, if all residues are removed from the field.

If residues are retained, soybean N budgets range from

-35 to 50 kg N ha^{-1} depending on the cultivar and environmental conditions (Sanginga et al., 2002). Amanuel et al., (2000) reported that negative N balance was due to the fact that nitrogen input (fertilizer and nitrogen fixation) was not enough to meet crop demand. They also demonstrated that N balance after legume harvest is positive when crop residues are returned to soil and only seed or grain is removed. The same results were reported (Adu-Gyamfi et al., 2007) for intercrops of maize-pigeon pea. They showed that, in situations where the aboveground biomass of both the legume and the cereal were removed from the field, high negative values were observed, but if only the grains were exported, all budgets were improved. However, Schipanski et al. (2010) found that a positive N balance resulted when the percentage of N from fixation was greater than 60%. Laberge et al., (2009) found the same result on soybean, but they showed that errors can arise when important processes are ignored and the results of their study suggested that N rhizodeposition from soybean and grain legumes was such a neglected process. Rhizodeposits include roots exudates, fine roots, and root necrosis products accrued in the soil during plant growth (Hertenberger and Wanek, 2004). In reality, most plant biomass, and its N content, left in the field from the previous cropping season is lost over the dry season due to strong winds, free-roaming ruminants, bush fires and termites (Schulz et al., 2001). Ghosh et al., (2007) showed that besides the direct addition of N through the above-ground biomass, the legumes may enhance the soil available N pool for following crops via root exudates or inefficiencies in recovering soil mineral N during the legume phase, and subsequent decomposition of root and nodule residues. Birouste et al., (2012) and Arcand et al., (2013) have estimated that root-derived N comprises as much as 80% of the total ground N. These indirect additions of N contribute to substantial saving of N (20 to 30 kg N/ha) and enrichment of soil fertility even when all the legume residues are removed (Sharma, 2005).

In West Africa, the use of dual-purpose grain soybean varieties with potential for good nitrogen fixation and nitrogen balance is a promising technology that has multiple benefits such as improving household nutrition, source of cash income, and supply of N inputs, which can contribute to improve soil fertility and to the sustainability of the cropping systems since farmers are often reluctant to adopt legume cover crops that are not useful for human consumption or without a direct economic benefit (Mayong et al., 1999; Vanlauwe et al., 2001). Thus, soybean with a positive N balance can be used to replenish soil nutrients and contribute to reduce land degradation occurring in Benin and consequently improve subsequent cereals crops. Indeed, the benefits of including legumes as green manures in rotations with cereal crops as a source of nitrogen (N) is well documented (Mason and Spaner, 2005; Kirkegaard et al., 2008; Peoples et al., 2009a). Legumes have the ability to supply a renewable source of N to agricultural soils through biological N-fixation, providing an economically and ecologically attractive means of delivering N to non-leguminous crops and reducing off-farm N inputs (Kirkegaard et al., 2008; Peoples et al., 2009; Thiessen Martens et al., 2001). However, smallholder farmers invariably carry the whole shoot of soybean from the fields for threshing. Or, if the legume stover is removed, there is often no observable benefit to the next crop and there is usually a net removal of N from the cropping system in the legume grain (Giller, 2001) and this is well reported in our results indicating negative N budget ranging from -91 to -17 kg N ha^{-1}.

Conclusion

This study aimed to assess biological nitrogen fixation of fourteen soybean varieties using isotopic dilution method with maize as reference plant. With this method, it appeared that all soybean varieties tested fixed nitrogen derived from air. But a particular problem of this method is that the enrichment the ^{15}N enrichment of the soil N would need to be relatively constant over time and space, or the time course and depth of soil N uptake by the reference and N_2-fixing plants the same. The result would be more reliable if a non-fixing soybean cultivar instead of maize was used, because soybean and maize do not have similar root characteristics.

The study showed that Canarana, TGX 1448 2E and BRS Paraiso soybean varieties were identified as the highest amount of N fixed capacity among the 14 varieties. From the results, it could be concluded that the involvement of Canarana, TGX 1448 2E and BRS Paraiso soybean varieties in cereal-based cropping systems would help reduce N inputs and improve soil and crop productivity in farming systems in Benin. The incorporation of legume residues into the soil immediately after harvest might enhance positive nitrogen balance in cropping systems.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Variation of static pressure in a crambe (*Crambe abyssinica* Hochst) grains column

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The crambe has emerged as an option for the production of biodiesel, constituting itself as an alternative for off-season grain in Brazil. To obtain the best performance in the post-harvest product processing, knowledge of physical characteristics of the grains to as well as of the design of machines and structure storage is necessary. After pre-cleaning, the crambe (*Crambe abyssinica* Hochst) with a water content of 8% (wb) was placed into a silo prototype, provided a fan and plenum, and subjected to five airflow densities that were pre-determined in a total of four repetitions for each tested airflow. The static pressure was measured through the column in five layers. The results show that there is a significant effect of air flow on the static air pressure in the crambe column, which increased linearly with depth. The experimental data fitted with good accuracy the models of Hunter and Shedd, thus enabling their use for the crambe column as well. The objectives of this study were to evaluate the variation of static pressure along a column of grains of crambe that were subjected to five airflow densities and check the fit of this variation following mathematical models suggested by Sheed and Hunter.

Key words: *Crambe abyssinica*, resistance to airflow, Sheed, Hunter.

INTRODUCTION

The crambe is a species native from the Mediterranean region belonging to the Brassicaceae family, of annual cycle, with seeds presenting an oil content ranging from 28 to 60%. Thus, the crambe becomes a promising crop for biodiesel production (Carneiro et al., 2009; Silva et al., 2013, 2014).

In Brazil, the crambe have been noted as a great option for the intercrop, because it is a winter crop that is characterized by both frost tolerance and drought resistance (Oliva et al., 2012).

The introduction of crambe in the country is recent. The first experiments indicated the year 1995 as the year that

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crambe was introduced. Therefore, there is a lack of information about the culture in Brazilian conditions, particularly technical issues related to post-harvest and for instance, their physical characteristics.

For the consolidation of crops in the country, the information about the drying and storage of the product becomes important, thus enabling post-harvesting structures to be built with technical parameters that are suitable for the crambe (Biaggioni et al., 2005).

The knowledge of the resistance to airflow in the grain bed is essential to design grain-drying and ventilation systems (Chun et al., 2011). This knowledge is also vital to select a ventilator that adequately provides the airflow that can overcome the pressure gradient supplied by grain (Abou-el-Hana and Younis, 2008).

The distribution of airflow in the grain mass depends on several factors, including the method of filling the grain, porosity, depth of grain yield, grain morphology and configuration, the air velocity, and impurity (Khatchatourian et al., 2009).

One can make some inferences about the static pressure. The greater the thickness of the grain mass, the higher the static pressure will be. Small grains offer more static pressure than big grains; lower water content in grains can increase the pressure to the passage of air. Therefore, experiments with low water levels have a higher safety margin in the formulation of projects.

In a mass of grains or seeds, the decrease of static pressure exhibited when traversed by airflow can be estimated by empirical curves, in which the static pressure is related to the airflow (Biaggioni et al., 2005). A frequently used model is a graph in logarithmic scale proposed by Shedd (1953) with a ratio to 22 types of grains. Another model used is proposed by Hunter (1983), who studied the static pressure difference through a mass of grains representing the Ergun model (1952) by the following equation:

$$\Delta P = MV + NV^2 \qquad 1$$

Where M and N represent the parameters of the fluid and the granular mass required for Ergun's formulation (1952).

A large number of studies exist with regard to airflow resistance of cereals, oilseeds, and vegetables, but no information about the crambe that exhibits resistance to an airflow of 33 crops exists in the literature or is even compiled (ASAE, 2011).

This study aimed at evaluating the variation of the static pressure along a column of grains of crambe with 8% water content that was submitted to five airflow densities; it also aimed at checking the fit of this variation by following the mathematical models suggested by Sheed and Hunter.

MATERIALS AND METHODS

This study was conducted at the Universidade Estadual Paulista (UNESP), Faculty of Agricultural Sciences, Campus of Botucatu/São Paulo. Crambe grains with 8% water content after pre-cleaning were placed into a silo prototype characterized by a galvanized steel column (with five taken for measuring the static pressure), plenum, and fan (Figure 1).

Crambe grains were placed in free fall within the prototype, reaching a height of 1 m. The test consisted of static pressure measurements by using an inclined tube differential pressure gauge (18°), Dwyer brand; 0.5 mm precision of water column, at different depths, for each of the four repetitions, totaling twenty measures of depth in the column.

The densities of airflows used were as follows: 4.76, 6.41, 8.51, 9.90, and 10.47 $m^3 min^{-1} m^{-2}$. The experimental design was completely randomized, with five lots of crambe and four replications. After obtaining the results, the averages were submitted to an analysis of variance and means were compared by the "t" test ($p \leq 0.05$).

For adjusting the equations proposed by Sheed (1953) and Hunter (1983), the parameters *a* and *b* were used in Equation 1 and M and N were used in Equation 2. These parameters emerge from the specific physiological characteristics of each type of grain, and it is necessary to obtain these parameters by using simple linear regression analysis.

In the determination of the best adjustment, the coefficient of determination (R^2) and the average percentage deviation (P) were used.

$$P = \frac{100}{n} \sum_{i=1} \left| \frac{\Delta P_{exp} - \Delta P_{calc}}{\Delta P_{exp}} \right|$$

where P is the average deviation in percentage,%; n is the amount of experimental data; Ap exp is the pressure gradient values obtained experimentally, Pa m^{-1}; and Ap calc is the pressure gradient values predicted by the model, Pa m^{-1}.

RESULTS AND DISCUSSION

The results show that there was a significant effect of all air flow densities, suggesting the influence of this variable in the resistance to airflow. The pressure drop is related to the increase in bulk density as well as reduction of the porosity of the mass of grains is promoted by raising the water content in the product (Amanlou and Zomorodian, 2011).

In the physical characterization of crambe, it was found out that the density of 347.36 kg m^{-3} corroborates with that of Silva et al. (2013) and Gonçalves et al. (2014). The porosity of crambe vary from 43 to 48% depending on the temperature and water content, corroborating the average for other grains, around 35 to 50% voids, this fact is due to the physical characteristics of the tegument crambe (Gonçalves et al., 2014).

The results of the variation of the static pressure gradient in function of air flux density are shown in Table 1. For the densities of airflow used (4.76 to 10.47 $m^3 min^{-1} m^{-2}$), the gradient of precision statics ranged from 165.0 to 427.5 m Pa^{-1}. The values showed that there was a significant effect between all densities of the airflow, which suggests the influence of this variable in the resistance to airflow.

The increase of the pressure drop from rising air flow can be attributed to the increase of kinetic dissipation as

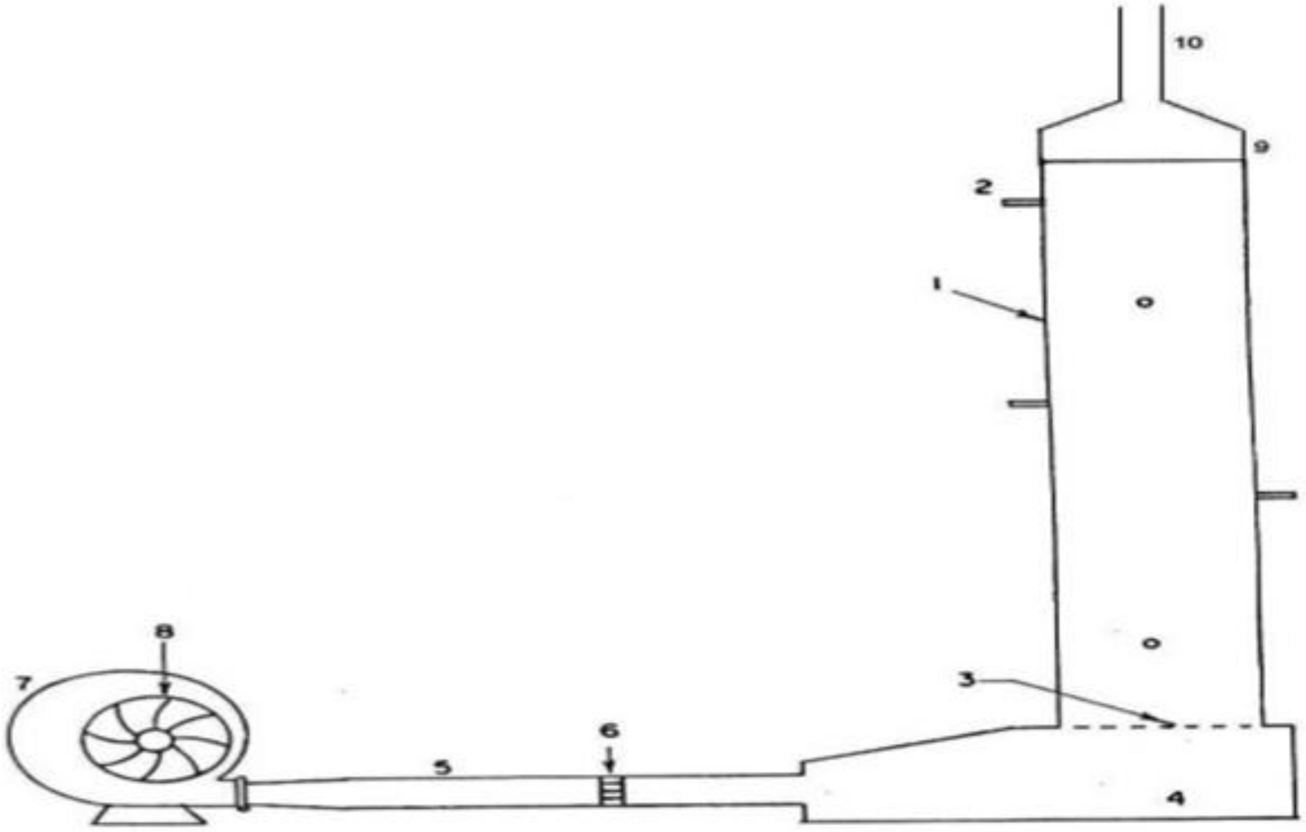

Figure 1. Scheme of the prototype used in determining the static pressure gradient of crambe. 1) Galvanized column, 1.20 m tall; circular section, 0.50 m in diameter; 2) Measuring the static pressure taps: copper pipes (5 mm in diameter) spaced 0.20 m vertically along the column; 3) Base perforated in metal with circular openings; 4) Chamber plenum: square section (0.55 × 0.55 m), 0.33 m high; 5) Tube with galvanized sheet metal connecting the fan to the plenum: 1.20 m long and 0.12 m in diameter; 6) Homogenizer airflow; 7) Centrifugal fan with straight blades: driven by an electric motor of 1/3 CV; 8) Diaphragm of air intake: allows one to control and vary the intake of airflow; 9). Cone air exit reducer; 10) Measuring to speed.

Table 1. Average values of static pressure gradient from crambe grains, in function on the airflow density.

Density of airflow (m^3 min^{-1} m^{-2})	Static pressure gradient (Pa m^{-1})
4.76	165.0^{a}
6.41	242.5^{b}
8.51	327.5^{c}
9.9	400.0^{d}
10.47	427.5^{e}
LSD	0.016
CV (%)	3.43

LSD: Least significant difference; CV: coefficient of variation. Means followed by the same letter do not differ, the “t” test ($p \leq 0.05$).

air speed increases (Agullo and Marenya, 2005).

However, the relationship between the air flow velocity and pressure gradient are different for each type of grain. This is due to factors such as, geometric shape of the grains, porosity, water content, compaction factor as others that provide differences in the roughness of the particle surface and thus alter the static pressure of the grains (Khatchatourian and Binello, 2008).

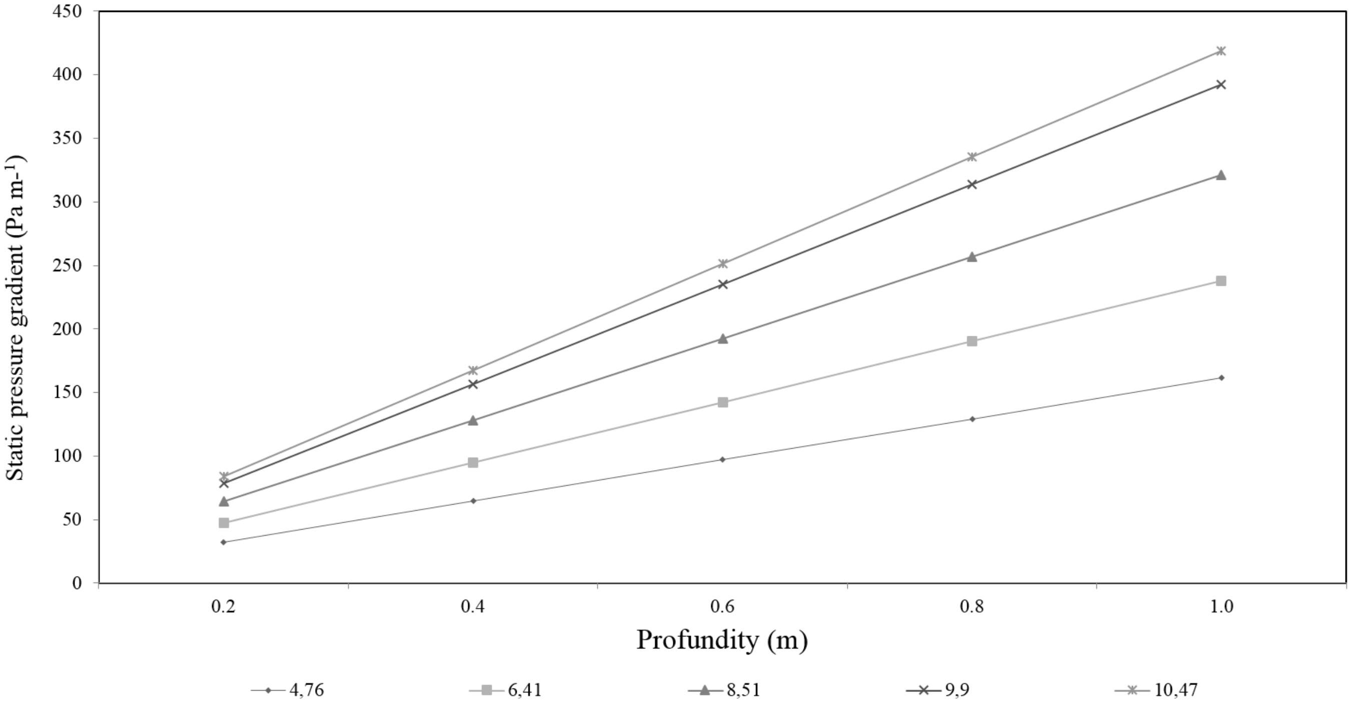

Figure 2. Effect from depth (m) and the density of the airflow (m^3 min^{-1} m^{-2}) on the static pressure gradient (Pa m^{-1}) in a column of crambe grains.

Table 2. Average percentage deviation (P) and coefficents of determination (R^2) from Shedd and Hunter models obtained by regression to the crambe grains.

Shedd	Hunter
P = 2.37%	P = 2.7%
R^2 = 98.0%	R^2 = 99.0%
a = 0.06876	M = 35.16
b = 0.82955	N = 0.6785
-	K = - 15.77

The variation in static pressure gradient measured in five layers and five airflow densities is as shown in Figure 2. Figure 2 establishes a dependency between the air flow velocity and static air pressure in the grain mass to varying depths of the storage layer. It is possible to look at a linear behavior of the static pressure curve with respect to depth from the crambe grain layer depicted. The increase in the static pressure of the gradient with the highest airflows is noted. These results corroborate those obtained in other experiments, such as quinoa (Gratão et al., 2013), peanut pods (Figueiredo et al., 2012), macadamia nut (Biaggioni et al., 2005), canola (Santos et al., 1999), white (Lukaszuk et al., 2008), grains, and cereals in general (ASAE, 2011).

According to Neethirajan et al. (2006) who have a seed compaction at the bottom of the silo. Possivelmete is one of the factors that causes signficant differences in static pressure drop at different depths, however, for Khatchatourian et al. (2009), simply increasing the depth of the grain mass can promote the increase of resistance to air flow, not necessarily being the increase in the degree of compaction in the deeper layers solely responsible for the increased resistance to air passage. The coefficients *a* and *b*, from Shedd model, and M and N, from Hunter model, are shown in Table 2. The coefficients of determination (R^2), 98% (Shedd) and 99% (Hunter), near 1 indicate a good adjustment and suggest good applicability of the two models for crambe culture. For the Hunter model, it is proposed to insert the constant K in the following equation:

$$\Delta P = K\,MV + NV^2$$

where K is a constant.

The model that has the best description of resistance to the air passage is one that has the highest coefficient of determination, and the lowest average percentage deviation (P) is recommended to be less than 5% (Kashaninejad and Tabil, 2009). The two settings used were below this recommendation in evaluating this parameter. The Shedd model was more suitable, because

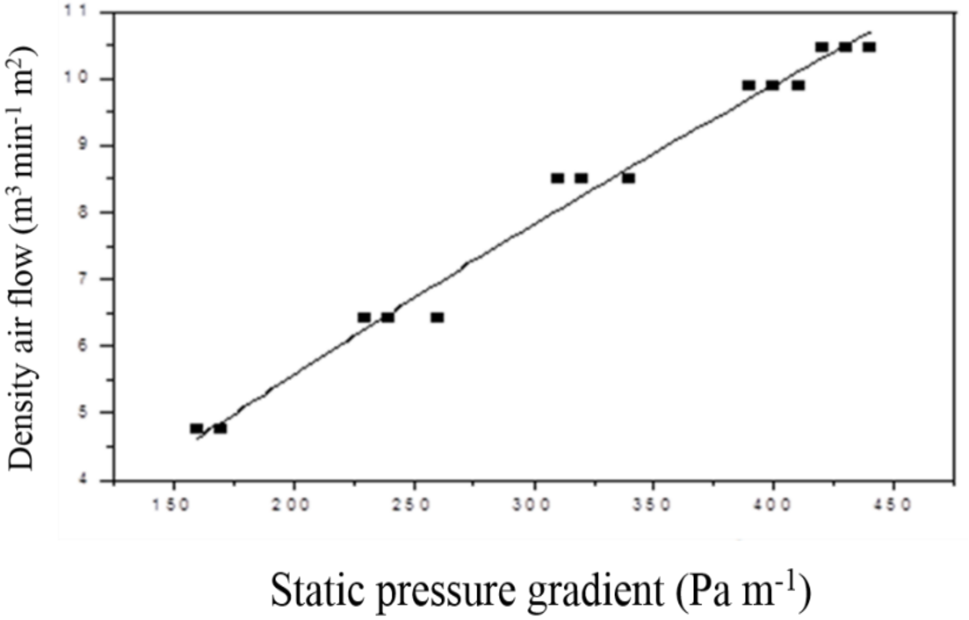

Figure 3. Comparison between the curves of the static pressure variation in the crambe grains column experimentally obtained by Shedd model.

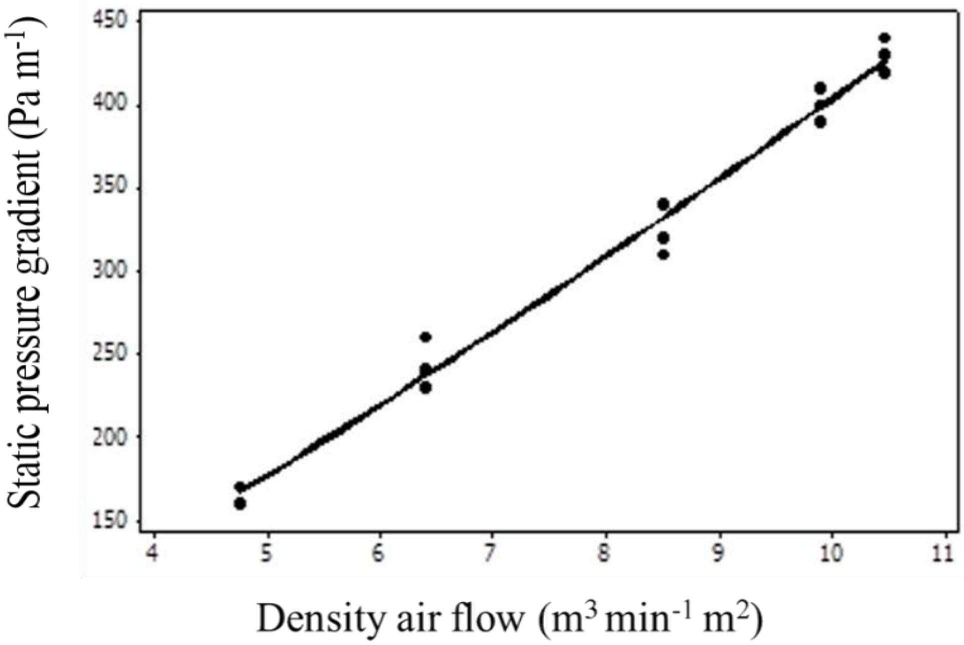

Figure 4. Comparison between the curves of the static pressure variation in the crambe grains column experimentally obtained by Hunter model.

it presented a lower value than the Hunter model.

The results corroborate research with other agricultural products such as canola (Andrade et al., 2001), quinoa (Gratão et al., 2013), and chickpeas (Shahbazi, 2011).

In Figures 3 and 4, the experimental data are shown as comparing the static pressure drop of the crambe grains column by using the Shedd and Hunter model. The results corroborate research with other agricultural products such as quinoa (Gratão et al., 2013), chickpeas (Shahbazi, 2011), and pistachio (Kashaninejad and Tabil,

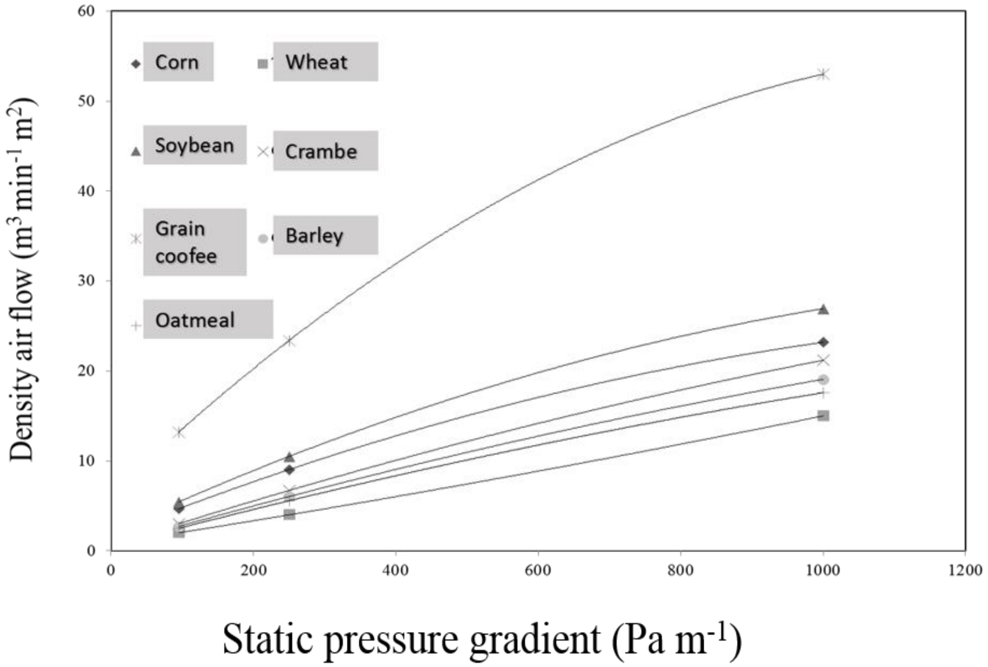

Figure 5. Pressure drop as a function of airflow for crambe grains with 8% water content compared with other grains.

2009), which point to the Shedd model as being the most suitable for determining the static pressure due to the higher coefficient of determination (R^2) compared with the Hunter model.

The Figure 5 shows the pressure loss in the function of air flux density with different grains in comparison to the crambe. It can be seen from Figure 5 that the static pressure exerted by crambe is greater than that of other grains like soy, corn, and coffee, and approximates to the resistance offered by small grains such as barley and oats; this fact is due to the size and shape of the crambe grains with characteristics that are very similar to these small grains (ASAE, 2011).

Conclusions

The static pressure increased linearly with an increase in air density and flow with an increase in depth of the vertical column of crambe. The variation of the static pressure provided by crambe, approaches from oat and barley. The Shedd and Hunter models adjusted satisfactorily to the experimental data in the airflow range investigated for crambe.

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Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Strategies for selecting soybean genotypes using mixed models and multivariate approach

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The objective of this study was to select soybean genotypes derived from crosses between conventional and transgenic lines Roundup Ready (RR), using jointly Restricted Maximum Likelihood/Best Linear Unbiased Prediction (REML/BLUP) approaches, factors analysis and principal components analysis, processed with favorable agronomic traits, during the 2013/2014 growing season. Three agronomic selection processes were identified to select genotypes that discriminate genotypes containing more specific properties. Process 1 (insertion height of first pod, HFP; number of branches, NB; number of pods, NP; number of nodes, NN; and grain yield, GY) was efficient to select earlier, smaller genotypes with good yield/production components and lodging resistance. The junction between mixed model via REML/BLUP and the applied multivariate statistic using factor analysis helped to select suitable genotypes with high performance to carry on the soybean plant-breeding program.

Key words: *Glycine max*, Restricted Maximum Likelihood/ Best Linear Unbiased Prediction (REML / BLUP), factor analysis, principal components.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill), with its expanding commercial crop areas, has become very important in the world scenario (Cavalcante et al., 2010). The species have a complex production, storage, processing and marketing structure, where they are grown on a large scale (Rezende and Carvalho, 2007).

In Brazil, soybean growth and productive capacity improving are tied to advances in breeding programs and environmental conditions (Klahold et al., 2006). Genetic improvement has contributed to increase soybean production, and genetic gains resulted from traditional methods involving hybridization and consequent phenotypic selection. Currently, it is combined with the use of transgenic and molecular markers (Peluzio et al., 2009).

One of the most important features of soybean breeding programs is searching for cultivars with favorable traits to obtain significant productivity gains.

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However, genetic gains have become increasingly difficult to achieve for species submitted to long selection processes (Maia et al., 2009).

More accurate statistical methodologies should be employed to obtain highly effective estimates of the genetic gain, which is expected in each selection cycle. Besides, plant breeding has a strong link with statistics, therefore, in addition to selection methods, good field trials, and relevant resources for choosing genetic designs, the more recent trend uses more refined statistical analytical procedures for a more detailed study of components of the average and variance of a character (Maia et al., 2009).

For many years, plant breeding programs depended on selecting genotypes by analyzing each agronomic variable individually, estimating genetic parameters, applying selection indices for traits and analyzing the environments to check the genotype x environment interaction. In recent years, multivariate methods and mixed models have become more important due to advances in computer software, and are being applied to evaluate genetic divergence (Costa et al., 2006; Oliveira et al., 2008; Bizari et al., 2014), select genotypes and progenies (Vianna et al., 2013; Dallastra et al., 2014), study adaptability and stability of genotypes (Maia et al., 2006; Mendonça et al., 2007; Borges et al., 2010b; Gomez et al., 2014), estimate genotypic values and study genetic parameters (Duarte et al., 2001; Lopes et al., 2008).

In view of the positive aspects of mixed models and multivariate analyses, this study aimed to select superior soybean genotypes that originated from crosses between conventional and transgenic lines (Roundup Ready®) (RR), using the REML/BLUP methods, factors analysis and principal components analysis.

MATERIALS AND METHODS

Genetic material, experimental site and agronomic traits

This study evaluated soybean segregating populations that resulted from crosses between conventional lines of the College of Agricultural and Veterinary Sciences/UNESP, Jaboticabal. These genotypes are widely adapted, and the commercial cultivars carry the RR gene of the MSOY RR group.

The trial evaluated 202 soybean genotypes from the generation F_6 during the 2013/2014 growing season, in the Experimental Farm of Education, Research and Production (FEPE), of Agricultural and Veterinary Sciences College/UNESP, Jaboticabal, SP.

The experimental design was augmented Federer blocks, containing 13 blocks, with 5 m long rows of plants spaced 0.45 m as the plots. Four standard cultivars were used as additional checks, two conventional ('CODETEC-216' and 'Vmax') and two carriers of the RR gene ('BMX-Força RR' and BRS 'Valiosa RR').

The following agronomic traits were evaluated in six plants per parcel, in beginning flowering stage (R1) till full flowering stage (R2): a) days to flowering (DF) - number of days elapsed from plant emergence up to when 50% of the flowers opened; b) Plant height at flowering (PHF) – the distance between plant insertion in the soil and the apex of the main stem, in centimeters (cm).

The following traits were evaluated at full mature stage (R8): a) days to maturity (DM) (Fehr and Caviness, 1977)- period elapsed between sowing and the date when 50% of the plants displayed 95% of mature pods; b) plant height at maturity (PHM) – distance measured on the stem between plant insertion in the soil and the insertion of the uppermost pod, (cm); c) insertion height of first pod (HFP) - distance between the soil surface and the insertion of the first pod (cm); d) Lodging (LD) - evaluated by a visual score, ranging from 1 (all plants standing) to 5 (all plants lodging); e) agronomic value (AV) - assessed by a visual score, ranging from 1 (plants with poor agronomic traits) to 5 (plants with optimal agronomic traits). The scores evaluated a set of visual adaptive traits: plant architecture, number of filled pods, vigor and plant health, premature pod threshing and leaf retention at maturity; f) Number of branches per plant (NB) - total number of branches attached to the main stem of the plant; g) Number of nodes (NN) – total number of internodes per plant; h) Number of pods (NP) - total number of pods with formed seeds per plant; i) Grain yield (GY) - grain weight per individual plant obtained after plant harvesting, processing and drying of the grains (up to 13% moisture), expressed as grams per plant (g plant $^{-1}$); j) Hundred seeds (grains) weight (HSW) – the average weight of four samples of hundred seeds determined using a precision balance (1 g).

Estimation of genetic parameters

Genetic parameters were estimated by restricted maximum likelihood (REML) and genotypic means (generation F_6) adjusted and estimated by Best Linear Unbiased Prediction (BLUP). In the analysis of mixed models with unbalanced data, the model effects are not tested via F tests as is done in the analysis of variance method. In this case, for the random effects, the scientifically recommended test is the likelihood ratio test (LRT) (Resende, 2007b). The likelihood ratio test (LRT) was used to evaluate the traits significance in the experiment which was determined by the chi-square at 5% and 1% probability with one degree freedom (Nelder and Wedderburn, 1972). Considering the experimental augmented blocks of Federer, the matrix data was analyzed according to the statistical model (Resende, 2007b):

$$\mathbf{y} = \mathbf{X}\mathrm{f} + \mathbf{Z}\mathrm{g} + \mathbf{W}\mathrm{b} + \mathbf{e}$$

Where: y is the data vector; f, vector of fixed effects (general average); g, vector of genotypic effects (assumed to be random); b, vector of block environmental effects (assumed to be random); e, vector of errors and residues (random); X, Z and W are the incidence matrices for these effects (f, g, ande, respectively).

The distribution and structures of means and variances are given below according to Barreto and Resende (2011):

$$E\begin{bmatrix} y \\ g \\ b \\ e \end{bmatrix} = \begin{bmatrix} Xf \\ 0 \\ 0 \\ 0 \end{bmatrix}; Var\begin{bmatrix} g \\ b \\ e \end{bmatrix} = \begin{bmatrix} I\sigma^2 & 0 & 0 \\ 0 & I\sigma^2 & 0 \\ 0 & 0 & I\sigma^2 \end{bmatrix}$$

The mixed model equations for the model adopted are (Barreto and Resende, 2011):

$$\begin{bmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z + I\lambda_1 & Z'W \\ W'X & W'Z & W'W + I\lambda_2 \end{bmatrix} \begin{bmatrix} \hat{f} \\ \hat{g} \\ \hat{b} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ W'y \end{bmatrix}$$

Where: $\lambda_1 = \frac{\sigma_e^2}{\sigma_g^2} = \frac{1-h_g^2-b^2}{h_g^2}$; $\lambda_2 = \frac{\sigma_e^2}{\sigma_b^2} = \frac{1-h_g^2-b^2}{b^2}$

h_g^2 = Heritability of individual parcels, b^2 = Determination coefficient of block effects, σ_g^2 = Genotypic variance between lines, σ_b^2 = Variance between blocks, σ_e^2 = Residual variance between parcels. Statistical analysis for mixed models was performed using the linear analysis procedure of the PROC MIXED software (SAS Institute, 2011).

Selection of genotypes

The genotypes were selected using exploratory multivariate statistical techniques due to the structure of dependence in the original set of variables. The multivariate technique known as factor analysis used the method of principal components, calculated from the correlation matrix. This study used varimax rotation method (Manly, 2008).

Each process is identified in the factor according to traits with the most representative loads (greater than 0.50). The processes identified in the factors are called agronomic selection processes. The traits considered in the processing of factor analysis were the genotypic averages estimated by BLUP, as follows: DF, PHF, DM, PHM, HFP, LD, AV, NB, NN, NP, GY and HSW in the studied F_6 generation.

The discrimination of genotypes was performed by principal component analysis taking into account all traits, followed by each individual case (Cruz et al., 2012). The Kaiser criterion (1958) was used to select the main components, those whose eigenvalues were above unity since they generate components with relevant amount of the original information.

Each graph displays two circles that resulted from the principal component analysis: a smaller one with diameter between -2 and 2 ($\alpha \approx 5\%$), and a larger one with diameter between -4 and 4 ($\alpha < 0.01$). Values located outside each circle were considered genotypes with properties specific for selection.

After standardizing the variables (mean = 0 and variance = 1), the analyses were performed using the STATISTICA Version 7 software (StatSoft, 2004).

RESULTS

The analysis of deviance, ANODEV (Nelder and Wedderburn, 1972), detected significant differences by Chi-square test (LRT) at 1% probability for the following agronomic traits: days to flowering (DF), plant height at flowering (PHF), plant height at maturity (PHM), insertion height of first pod (HFP), number of nodes (NN), number of pods (NP), grain yield (GY) and hundred seeds weight (HSW) (Table 1). GY and NP showed greater variations (LRT = 62.9 and LRT = 53.3, respectively). However, the agronomic traits, days to maturity (DM), lodging (LD), agronomic value (VA) and number of branches (NB) were not significant by chi-square at 5% probability.

The genetic parameters and genotypic means of the traits estimated by REML/BLUP indicated that the coefficients of genetic (CV_g) and environmental (CV_e) variation ranged from 0.83 to 92.0% and 6.70 the 69.5%, respectively (Table 2). The CV_g/CV_e ratio was greater than one for the following traits: NDF, PHF, PHM, HFP, NN, NP, GY and HSW. However, this ratio was not greater than one for traits that were not found significant by the analysis of deviance (chi-square test): DM, LD, AV and NB.

The estimated heritability coefficients (h^2) were low for all studied traits, which is undesired in the breeding program. Overall, the isolated variables of this study had either little or no variability to characterize a genotypic selection, and very low heritability estimates, considering each one individually, especially the important soybean agronomic traits of (DM, AV and LD). Consequently, genetic gains were low due to the fact that the studied population had undergone various selection processes, making it difficult to select for genotypes selection index. Nevertheless, factor analysis and principal components identified specific and important genotypes for breeding program.

Data at Table 3 showed the results of factor analysis while three agronomic processes with distinct patterns in the selection of genotypes were characterized, according to the suitability of the information traits acting together in the process.

The first factor (F1), accounting for 29.08% of the original variability, identified a process which aggregated only production traits. In this process, NP, NB and GY were inversely correlated with HFP. The second factor (F2), accounting for 29.74% of the remaining variability, aggregated the traits DF, PHF, DM, PHM and NN, associated with plant cycle and size, which were directly correlated. The third factor (F3), accounting for 12.77% of the remaining variability, aggregated the traits HSW (yield component), LD (lodging), which were directly correlated, but inversely correlated with VA (visual score of genotype quality).

Principal component analysis of Process 1 formed by HFP, NP, NB and GY, which discriminated genotypes regarding grain production was presented by Figure 1. In PC1, genotypes located outside the large circle to the left have higher yield, although displaying lower HFP (1, 50, 88, 165, 171, 172, 189 and 196) contrasting with the genotype 36, located to the right which displayed lower yield and greater insertion height of the first pod.

Data in Figure 2 showed that the second process (DF, PHF, DM, PHM and NN), to the right of PC1, outside the larger circle, characterized genotype 126, with higher PHF and DM, contrasting with genotype 152, located on the left near the zero reference line of PC1. Genotype 184 also located on the outer region of the circle, characterized by lower DF, PHF and DM and with greater PHM and NN. The goal was to determine earlier genotypes with height ranging from 0.80 to 1.0 m and the results indicated that genotype 152 is the closest to the ideal.

Figure 3 results indicated that genotypes 4, 26, 47, 56,78, 94, 101, 112, 119 and 139, which located in the region between the two circles to the left of PC1, had

Table 1. Analysis of deviance (ANODEV) of the agronomic variables evaluated in the studied soybean populations of generation F_6. Jaboticabal, SP, 2013/2014.

	DF $^{(a)}$			**PHF $^{(b)}$**	
Effect	**Deviance**	**LRT**	**Effect**	**Deviance**	**LRT**
Genotype	1571.9 $^{+}$	8.6 **	Genotype	1868.1 $^{+}$	7.7 **
Model	1563.3 $^{++}$		Model	1860.4 $^{++}$	
	DM $^{(c)}$			**PHM $^{(d)}$**	
Effect	**Deviance**	**LRT**	**Effect**	**Deviance**	**LRT**
Genotype	1681.9 $^{+}$	0	Genotype	1986.2 $^{+}$	21.5 **
Model	1681.9 $^{++}$		Model	1964.7 $^{++}$	
	HFP $^{(e)}$			**LD $^{(f)}$**	
Effect	**Deviance**	**LRT**	**Effect**	**Deviance**	**LRT**
Genotype	1430.1 $^{+}$	11 **	Genotype	532.5 $^{+}$	0
Model	1419.1 $^{++}$		Model	532.5 $^{++}$	
	AV $^{(g)}$			**NB $^{(h)}$**	
Effect	**Deviance**	**LRT**	**Effect**	**Deviance**	**LRT**
Genotype	605.4 $^{+}$	3	Genotype	954 $^{+}$	0
Model	602.4 $^{++}$		Model	954 $^{++}$	
	NN $^{(i)}$			**NP $^{(j)}$**	
Effect	**Deviance**	**LRT**	**Effect**	**Deviance**	**LRT**
Genotype	1182.8 $^{+}$	14.5 **	Genotype	2347.5 $^{+}$	53.3 **
Model	1168.3 $^{++}$		Model	2294.2 $^{++}$	
	GY $^{(l)}$			**HSW $^{(m)}$**	
Effect	**Deviance**	**LRT**	**Effect**	**Deviance**	**LRT**
Genotype	1765.3 $^{+}$	62.9 **	Genotype	1018.3 $^{+}$	21.3 **
Model	1702.4 $^{++}$		Model	997 $^{++}$	

$^{(a)}$ days to flowering; $^{(b)}$ plant height at flowering (cm), $^{(c}$ days to maturity; $^{(d)}$ Plant height at maturity (cm); $^{(e)}$ first pod insertion height (cm), $^{(f)}$ Lodging; $^{(g)}$ agronomic value; $^{(h)}$Number of branches, $^{(i)}$ Number of nodes, $^{(j)}$ Number of pods; $^{(l)}$ Grain yield (g.plant^{-1}), $^{(m)}$ hundred seeds weight (g.plant^{-1}). ** LRT (likelihood ratio test) - Chi-square tabulated: 3.84 and 6.63 for the significance levels of 5% and 1%, respectively. $^{+}$ Deviance of the fitted model without effect of genotype. $^{++}$ Deviance of the fitted model with effect of genotype.

more specific traits: more lodging, higher HSW and lower visual scoring. Among them, Genotypes 4 and 139 were highlighted. In contrast, Genotypes 31, 36, 40, 79, 126, 147 and 169 had good visual traits, low LD, but lower HSW. Genotype 118 had high HSW, lower AC, but low AV.

A principal component analysis without separation process had been performed with all variables to seek for specific genotypes (Figure 4). In PC1 Genotypes 36, 37, 126 and 170 which differentiated in the outer region of the larger circle, were characterized by greater DF, PHF, DM, PHM, NN, LD, HFP and AV in addition to lower grain yield. In contrast, Genotypes 1, 47, 49, 189, 50, 88, 152, 165, 171, 172, 183 and 196 displayed higher grain yield and lower HFP and AV. Moreover they were earlier, shorter and more resistant to lodging.

DISCUSSION

The significant differences of DF, PHF, PHM, HFP, NN, NP, GY and HSW detected by ANADEVI indicated a high variability among studied population. They also indicated that the variance components and their respective coefficients of determination were significantly different from zero in agreement with Resende (2007a).

However, the non-significance of: DM, LD, AV and NB may indicate a narrowing of genetic variation as a result of lower divergence between the parents, being little contrasting to the characteristics analyzed. LRT equal to zero was observed for DM, LD and NB, which corresponded to a lack of genetic variability.

The variability of DF trait may be explained by the presence of early and late cultivars in the preparation of

Table 2. Genetic parameters and descriptive statistics of agronomical traits evaluated in studied soybean populations of the F_6 generation. Jaboticabal, SP, 2013/2014.

Variance components [(a)]	Agronomic variables [(b)]											
	NDF	PHF	NDM	PHM	HFP	LD	AV	NB	NN	NP	GY	HSW
σ_g^2	21.8	74.9	0.9	191.6	13.3	0.02	0.23	0.02	5.71	1080.5	95.4	2.92
σ_e^2	18.6	66.2	57.0	57.4	9.2	0.44	0.42	2.67	2.51	101.8	66.0	1.14
σ_p^2	40.4	141.1	57.9	249.1	22.5	0.45	0.66	2.69	8.23	1182.3	102.0	4.06
σ_b^2	2.32	10.54	7.6	10.92	1.98	0.04	0.0002	0.21	0.26	9.10	1.81	0.09
CV_g	12.3	10.48	0.83	14.13	24.20	9.46	13.08	6.69	16.58	68.52	92.0	12.3
CV_e	11.3	9.9	6.7	7.7	20.2	50.6	17.6	69.5	11.0	21.0	24.2	7.7
CV_g / CV_e	1.08	1.06	0.12	1.83	1.20	0.19	0.74	0.10	1.51	3.26	3.80	1.6
GSD	4.67	8.66	0.93	13.84	3.64	0.12	0.48	0.16	2.39	32.87	9.77	1.71
h^2 (%)	27.0	26.54	0.75	38.47	29.50	1.69	17.77	0.46	34.73	45.69	36.0	46.8
Average	38.1	82.58	112.7	97.97	15.05	1.31	3.70	2.35	14.42	47.97	10.6	13.9

[(a)] σ_g^2 = Genotypic variance; σ_e^2 = Environmental variance; σ_p^2 = Phenotypic variance; σ_b^2 = Environmental variance between blocks; CV_g = genetic variation coefficient; CV_e = environmental variation coefficient; CV_g / CV_e = genetic variation of environmental coefficient ratio; GSD = genetic standard deviation; h^2 (%) = Heritability. [(b)] DF = days to flowering; PHF = plant height at flowering (cm); DM = days to maturity; PHM = Plant height at maturity (cm); HFP = insertion height of first pod (cm); LD = lodging; AV = agronomic value; NB = number of branches; NN = Number of nodes; NP = number of pods; GY = Grain yield (g.plant^{-1}); HSW = hundred seeds weight (g.plant^{-1}); PROD = productivity (kg ha^{-1}).

Table 3. Factors and their factor loadings after rotation of the factorial axis using the Varimax method for studied traits in soybean populations of generation F_6. Jaboticabal, SP, 2013/2014.

Agronomic variables	Factor loadings after rotation *		
	F1	F2	F3
Insertion height of the first pod (HFP; cm)	.5769	.2407	0.0187
Number of pods (NP)	-0.9209	0.1039	.0882
Number of branches (NB)	-0.7949	-0.2283	0.0048
Grain yield (GY; g.plant^{-1})	-0.8676	-0.1302	.1318
Days to flowering (DF)	.1668	.7458	-0.1196
Plant height at flowering (PHF; cm)	.2788	0.674	-0.1585
Days to maturity (NDM)	0.208	.5556	0.225
Plant height at maturity (PHM; cm)	0.1294	.8198	-0.038
Number of nodes (NN)	-0.1803	.7597	.0621
Hundred seeds weight (HSW; g.plant^{-1})	.1565	-0.2682	.5449
Lodging (LD)	-0.1747	.3775	.6284
Agronomic value (AV)	.2998	.2314	-0.6812
Explained variance (%)	29.08	29.74	12.77

* F1 = first factor; F2 = second factor; F3 = third factor.

the crossings. However, DM is as important as DF, which did not vary significantly. It is noteworthy that, the estimates of flowering date and other soybean growth stages are highly relevant for culture management, and for growth and yield modeling. This information can assist crop management under adverse conditions, such as lack of water and lodging (Rodrigues et al., 2001). Therefore, according to the climatic conditions of the region, it is possible to stagger planting and harvesting (Almeida et al., 2011).

Evaluation of PHF is associated with searching for earlier cultivars with good productivity. Genotypes with the greatest height at flowering tend to have higher productivity and shorter cycles when accompanied by lower DF. Carvalho et al. (2002) reported that this trait may help to select for yield, and being very effective in the selection of more productive strains. Moreover, they also noted that PHM displayed positive correlation with

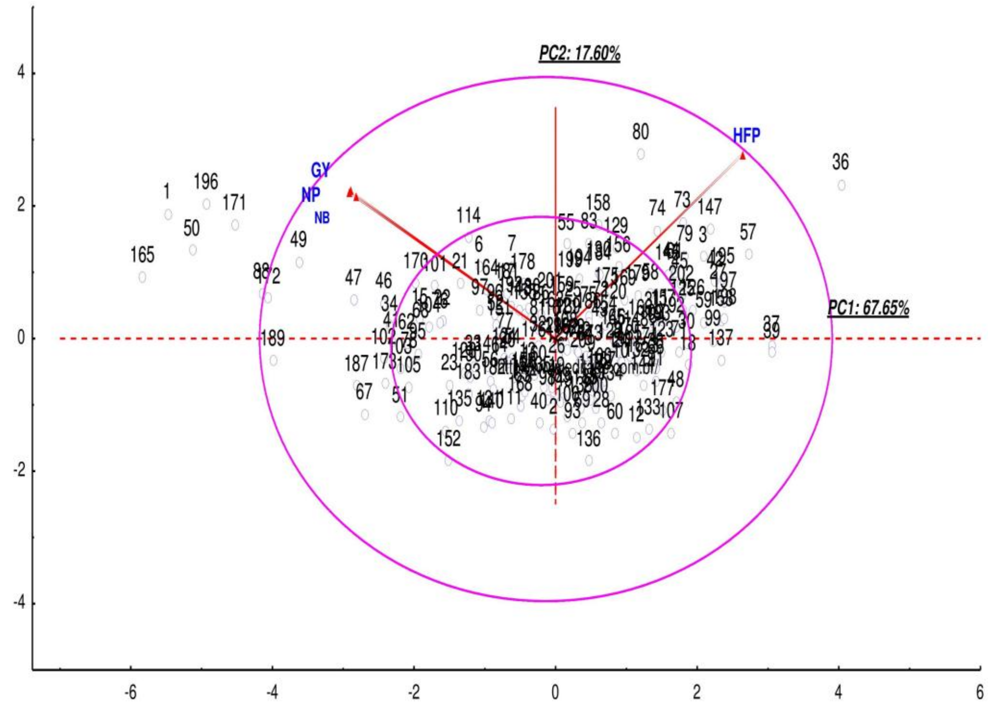

Figure 1. Principal component analysis of the first selection process for agronomic traits (HFP, NP, NB and GY) which discriminates genotypes regarding grain production, in soybean populations of the F_6 generation.Jaboticabal, SP, 2013/2014.

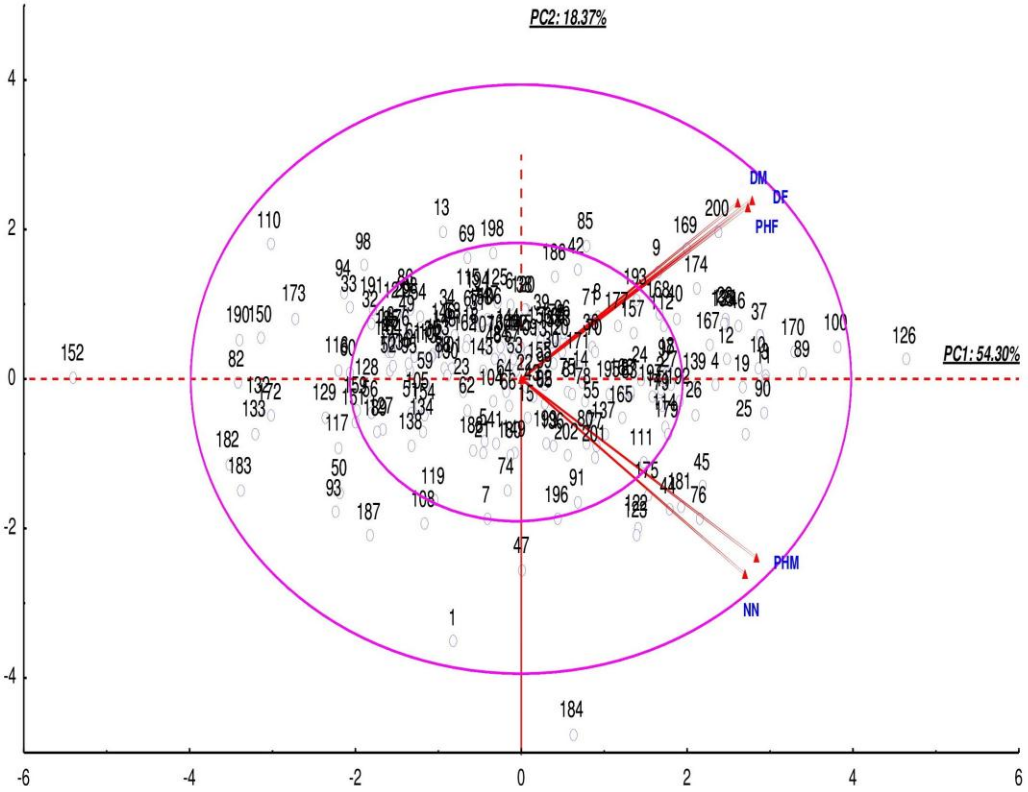

Figure 2. Principal component analysis of agronomic traits of the second selection process, in soybean populations of the F_6 generation.Jaboticabal, SP, 2013/2014.

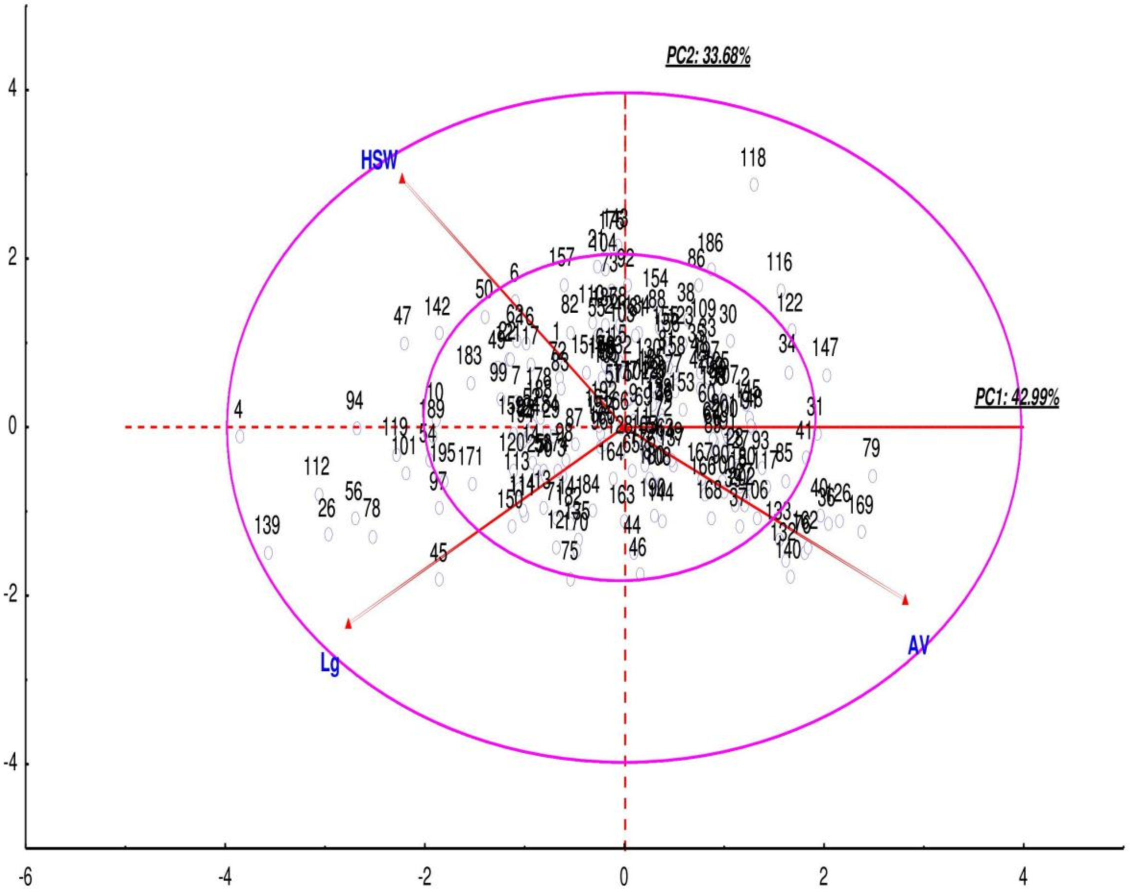

Figure 3. Principal component analysis of the traits of the third agronomic selection process, in soybean populations of the F_6 generation.Jaboticabal, SP, 2013/2014.

productivity, but PHF showed slightly higher correlation values with productivity.

Furthermore, the ideal HFP of soybean crops, under most conditions, is about 15.0 cm, although most modern harvesters can harvest well when the first pod insertion is as low as 10.0 cm (Rocha et al., 2012). However, this trait did not show significant correlation with grain yield (Muniz et al., 2002).

In addition, there is positive correlation between the PHM and LD. Buezzello et al. (2013) observed that the reduction of height of soybean plants was strongly associated to the lodging reduction, contributing to the increase in grain yield of the crop.

Studies have shown that NN and NP display positive correlation with grain yield (Muniz et al., 2002; Arshad et al., 2006; Dalchiavon and Carvalho, 2012), also contributing to indirect selection of genotypes.

The traits GY and HSW are highly correlated (Arshad et al., 2006), where GY is the plant individual output and HSW is related to the vigor of seeds and consequently of the plant, and being a production/yield component, as well.

Regarding to the studied traits, the ideal genotype sought should have high GY and AV. It should be earlier (lower DF and DM), resistant to lodging (LD = 1) while PHM should range from 0.80 to 1.0 m, and PHF higher or equal to 10.0 cm. The other studied traits (NDF, PHF, NB, NN, NP and HSW) are expected to enhance the selection of genotypes through correlations with more important genotypic traits.

The low values obtained for the CV_g/CV_e ratio when studying the genetic parameters may indicate lower experimental precision or higher number of genes controlling the trait. The fact that they are smaller than unity indicates unfavorable conditions for the selection of genotypes for these traits (Mistro et al., 2004). The heritability (h^2), on the other hand, shows potential for selection within experiments (Borges et al., 2010a). However, the values of h^2 in this study were obtained by REML, which avoids the overestimation of h^2.

The desirable characteristics for a soybean breeding program, in addition to selecting the most productive genotypes, are the earlier genotypes and heights that do not cause lodging. However, if inadequate tools are used there is the risk of selecting genotypes with poor agronomic traits, such as lower AV scores and lower pod

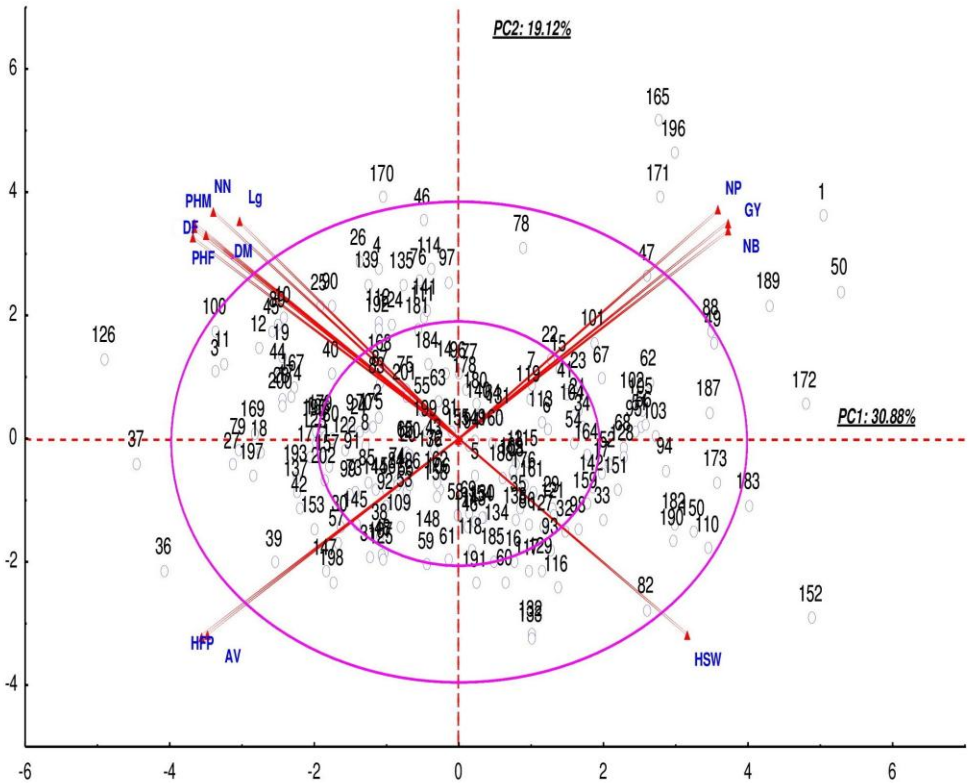

Figure 4. Principal component analysis of traits in soybean populations of the generation F_6.Jaboticabal, SP, 2013/2014.

insertion heights, which are not desired.

Conclusions

The characteristics number of days to flowering, plant height at flowering, plant height at maturity, insertion height of first pod, number of nodes, number of pods, grain yield and hundred seeds weight are suitable for the selection process, once they showed high genetic variability. Three agronomic selection processes were identified to select genotypes that discriminate genotypes containing properties that are more specific.

The selection strategy containing the variables insertion height of first pod, number of branches, number of pods, number of nodes and grain yield allowed the selection of soybean genotypes with good yield components, more early, smaller sizes and lodging resistance.

The junction between mixed model via REML/BLUP and the applied multivariate statistic using factor analysis helped to select suitable genotypes with high performance to carry on the soybean plant-breeding program.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Cultivation of colored cotton irrigated with saline water under potassium and nitrate/ammonium fertilization

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This study aimed to evaluate growth and production components of the colored cotton cultivar 'BRS Topázio' irrigated with saline water as a function of dose of potassium and proportions of nitrate and ammonium. The study was conducted in drainage lysimeters under greenhouse conditions, in a eutrophic Gray Argisol of sandy clay loam texture, in the municipality of Campina Grande, Brazil, from November 2014 to March 2015. A randomized block design was used to test five doses of potassium-DK (50, 75, 100, 125 and 150% of the recommendation for pot experiments) and five proportions of nitrate and ammonium-PNA (100/0, 75/25, 50/50, 25/75 and 0/100 mg of N kg^{-1} of soil). The K dose referring to 100% corresponded to 150 mg of K_2O kg^{-1} of soil. N supply in NO_3^-/NH_4^+ proportion of 25/75, promoted increase in growth variables (plant height, stem diameter and leaf area) and mass of cotton bolls. The K dose of 145.5 mg of K_2O kg^{-1} of soil promoted the formation of higher number of bolls plant^{-1}. The application of K in the doses of 112.5 and 187.5 mg of K_2O combined with 25/75 and 0/100 mg of NO_3^-/NH_4^+ promoted increment in cottonseed mass and the dose of 187.5 mg of K_2O associated with 25/75 mg of NO_3^-/NH_4^+ increased the number of days for flower bud opening.

Key words: *Gossypium hirsutum* L., saline stress, mineral nutrition.

INTRODUCTION

Belonging to the Malvaceae family, cotton (*Gossypium hirsutum* L.) is an oil seed crop cultivated in many regions of Brazil and worldwide, due to the versatility of its production. It is the main raw material for the textile industry, because of the characteristics and utilities of its fiber, and it is also used for the production of oil and other byproducts (Viana, 2014), constituting an important option for the economy, both for enabling labor force to remain in rural areas and creating jobs.

Cotton is one of the major agricultural, industrial and

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commercial commodity in various parts of the world. Cotton contributes significantly to Brazil's agricultural output and foreign exchange earnings. In terms of global production, China, USA, India, Pakistan and Uzbekistan are the world's major cotton producing countries, accounting for nearly 60% of the world production (FAOSTAT, 2015). In Brazil cotton production in the crop year 2013/14 was estimated at 7.2 million bales from an estimated area of approximately of 1.1 million hectares, representing a 20% increase in comparison to 2012/13 in production as well as planted area (CONAB, 2014).

The scarcity of water resources in the semi-arid region of Northeast Brazil and high salinity of water sources have led to the search of alternatives for the more efficient and the rational use of waters considered as of low quality (Alves et al., 2011). In this region, the use of brackish water can be an option for the production of crops like cotton, considering that this species tolerates high salt concentrations in the root zone (Oliveira et al., 2009, 2012, 2013; Sousa et al., 2010).

The use of water with salt problems can limit plant growth and production, due to the reduction in the osmotic potential of the soil solution, and also cause ionic toxicity, nutritional imbalances or both, due to the excessive accumulation of chloride and sodium (Flowers, 2004). However, plant sensitivity to salinity varies among species, cultivars of the same species and according to some factors like salt type and concentration, exposure time, phenological stage of the crop and availability of nutrients such as N and K, as well as the interaction between both (Ashraf and Harris, 2004).

Nitrogen can be found especially in the forms of nitrate and ammonium in the soil solution; however, relative absorption rates of nitrate and ammonium by higher plants are influenced by factors like the proportion of NO_3^-/NH_4^+ in the medium, temperature, carbohydrate concentration in the roots, salt concentration, among others (Taiz and Zeiger, 2009). In addition, the interaction between nitrogen sources (nitric and ammoniacal) has different effects on plant growth and development (Masclaux-Daubresse et al., 2010).

The supply of N as NO_3^- can result in decrease of dry matter production in plants with low capacity to reduce nitrate, because, in order to develop its functions in the plants, N needs to be reduced and incorporated into organic compounds (Ali et al., 2007). On the other hand, high levels of ammonium in cell tissues can be toxic and cause negative effects on root and shoot growth (Hachiya et al., 2012), causing physiological and nutritional disorders (Holzschuh et al., 2009).

Furthermore, the importance of the interaction between nitrogen (NO_3^-/NH_4^+) and potassium and the adequate proportion between both in the soil should be considered, because they are limiting factors in the processes of plant growth and development (Xu et al., 2002; Viana and Kiehl, 2010), since higher K absorption allows a rapid NH_4^+ assimilation, maintaining its content low in the plant and avoiding toxicity. However, in K-deficient plants, there is accumulation of NH_4^+, with the appearance of lesions corresponding to toxicity by this ion (Ajayi et al., 1970; Dibb and Welch, 1975).

In addition, K plays a key role in osmotic regulation and promotes the maintenance of turgor in guard cells, through the decrease of their osmotic potential, which results in higher water absorption by these cells and, consequently, it leads to greater turgor and stomata opening (LANGER et al., 2004). Thus, this study aimed to evaluate growth and production components of the cultivar 'BRS Topázio' of colored cotton irrigated with saline water as a function of potassium and different proportions of nitrate and ammonium fertilization.

MATERIALS AND METHODS

The experiment was carried out from November 2014 to March 2015 in pots adapted as drainage lysimeters in a greenhouse, at the Center of Technology and Natural Resources of the Federal University of Campina Grande (CTRN/UFCG), located in the municipality of Campina Grande-PB, Brazil (07°15'18'' S; 35°52'28'' W; 550 m).

The experiment was set in a completely randomized block design in a 5 x 5 factorial scheme, with three replicates. The treatments consisted of five proportions of nitrate and ammonium (NO_3^-/NH_4^+) (100/0, 75/25, 50/50, 25/75 and 0/100 mg of N kg^{-1} of soil) and five doses of potassium-DK (50, 75, 100, 125 and 150% of the recommendation for pot experiments, according to Novais et al., 1991). The recommendation of K dose referring to 100% corresponded to 150 mg of K_2O kg^{-1} of soil.

Cotton plants, cv. 'BRS Topázio', were irrigated using water with electrical conductivity (ECw) of 6.0 dS m^{-1}, and equivalent proportion of 7:2:1 of Na:Ca:Mg, which is the predominant relation in the waters used for irrigation in the semi-arid region of Northeast Brazil (Medeiros et al., 2003).

Irrigation water was prepared adding NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ salts in the water of the local supply system of Campina Grande-PB, considering the relation between ECw and salt concentration (10*$mmol_c$ L^{-1}=ECw dS m^{-1}), according to Richards (1954). After preparation and ECw calibration, using a portable conductivity meter, the saline water was stored in 200-L plastic pots, properly protected in order to avoid evaporation.

Drainage lysimeters with capacity for 20 L were used for plant cultivation. Each lysimeter was perforated at the bottom to allow drainage, and a transparent drain with diameter of 4 mm was installed at its base. The tip of the drain inside the lysimeter was involved with a non-woven geo textile (Bidim OP 30), in order to avoid obstruction by soil material. A plastic bottle was placed below each drain for the collection of the drained water to permit estimation of water consumption by plants.

Each lysimeter was filled with a layer of 0.5 kg of crushed stone, followed by 26 kg of soil material from a eutrophic Gray Argisol of sandy clay loam texture, from the district of São José da Mata (Campina Grande, Paraíba). Before filling the lysimeters, the soil was sampled for the determination of chemical and physico-hydric characteristics (Table 1) at the Laboratory of Irrigation and Salinity of the CTRN/UFCG, according to the methodology proposed by Claessen (1997).

The cultivar 'BRS Topázio' of colored cotton was used in this study. With light brown fiber, 'BRS Topázio' is derived from the crossing between the cultivars Suregrow 31 and Delta Opal. This cultivar stands out for having high fiber percentage (43.5%), high

Table 1. Chemical and physical characteristics of the soil used in the experiment.

Chemical characteristics									
pH (H_2O) (1:2.5)	OM dag kg^{-1}	P (mg kg^{-1})	K^+	Na^+	Ca^{+2}	Mg^{+2}	Al^{+3}	H^+	ECse (dS m^{-1})
			(cmol$_c$ kg^{-1})						
5.10	1.27	20.05	0.07	0.38	1.52	1.95	0.40	7.78	0.20

Physical characteristics									
Granulometric fraction (g kg^{-1})			Textural class	AD (kg dm^{-3})	PD (kg dm^{-3})	Total porosity (m^3m^{-3})	Water content (kPa)		AW
Sand	Silt	Clay					33.42	1519.5 (dag kg^{-1})	
528.50	202.00	269.50	SCL	1.14	2.71	57.93	14.00	4.87	9.13

OM – Organic matter: Wet digestion Walkley-Black; $Ca^{+2}$and Mg^{+2} extracted with 1 mol L^{-1}KCl at pH 7.0; Na^+ and K^+ extracted using 1 mol $L^{-1}NH_4OAc$ at pH 7.0; ECse- Electrical conductivity of soil saturation extract; SCL - sandy clay loam; AD –apparent density; PD- particle density; AW – available water.

uniformity (85.2%) and high resistance (31.9 gf/tex), which confers excellent characteristics, comparable to white-fiber cultivars and superior to the other colored-fiber cultivars. The mean yield of 'BRS Topázio', under irrigated conditions is 2,825 kg ha^{-1} (EMBRAPA, 2011).

Before sowing, the soil in all the lysimeters was brought to field capacity using respective saline water as per treatment. After seeding, irrigation was performed daily, by applying in each lysimeter the water volume necessary to maintain water content in soil close to field capacity. The volume was applied manually according to plant water demand, which was estimated through water balance: applied volume minus volume drained in the previous irrigation. In order to avoid salt accumulation in the root zone, a leaching fraction of 0.10 was applied every 15 days. Eight seeds of 'BRS Topázio' cotton were sown in each lysimeter at a depth of 2 cm and equidistantly distributed. At 15 and 25 days after sowing (DAS), thinning was performed in order to leave only one plant per lysimeter.

Basal dose equivalent to 300 mg of P_2O_5 kg^{-1} of soil based on the recommendation of Novais et al. (1991) was applied. Calcium nitrate, ammonium chloride and potassium chloride were used as source of NO_3^-, NH_4^+ and K^+, respectively. One third of the dose for each nutrient was applied at 15 DAS and the rest in three equal parts, applied together with irrigation water at intervals of 15 days. In order to avoid nitrification of ammoniacal N, together with ammonium chloride a nitrification inhibitor (dicyandiamide) in the dose equivalent to 10% of N was applied.

Cotton growth was evaluated at 30 and 130 DAS through plant height (PH), stem diameter (SD) and leaf area (LA). Production components were measured through the number of days for flower bud opening (NFBO), number of bolls per plant (NBP), cottonseed mass (CSM) and mass of one boll (M1B). Plant height was considered as the distance between plant base and the apical meristem. Stem diameter was measured at 5 cm from the plant base. Leaf area was obtained by measuring the midrib length of all the leaves in the plants, taking into consideration the methodology described by Grimes and Carter (1969), according to Equation 1:

$$y = 0.26622\ x^{2.3002} \qquad (1)$$

where y is the leaf area and x the midrib length of the main leaf of cotton. Total leaf area was determined by the sum of the leaf area of all the leaves.

The number of days for flower bud opening was determined through daily observations of flower appearance. After harvest (at 150 DAS), the number of bolls per plant was determined. CSM and M1B were measured using an analytical scale with precision of 0.01 g.

The data were subjected to analysis of variance by F test; when significant, regression analysis for potassium doses and test of Tukey for comparison of means at 0.05 probability level for the proportions of NO_3^-/NH_4^+were performed using the statistical program SISVAR-ESAL.

RESULTS AND DISCUSSION

According to the summary of the analysis of variance (Table 2), there was a significant effect ($p<0.05$) of the factor PNA on plant height, stem diameter and leaf area in the evaluated periods. As to DK and the interaction between factors (PNA x DK), there was no significant effect ($p>0.05$) for any of the analysed variables.

Plant height of 'BRS Topázio' cotton varied significantly ($p<0.05$) as a function of different studied proportions of nitrate/ammonium at 30 and 130 DAS (Figure 1A and 1B). Plants subjected to fertilization with proportions of NO_3^-/NH_4^+ of 25/75 mg significantly differed from plants fertilized with 0/100 and 100/0 of NO_3^-/NH_4^+. At 130 DAS growth of cotton was lower for the nitrate source, in comparison to the ammoniacal one. The reason why the ion NO_3^- (100/0 mg) contributed, at least partially, to this lower growth in the plant height, compared to 25/75 mg, can be explained by the high energetic cost for absorption and reduction of nitrate in plant metabolism (Guo et al., 2007), since after its passage through the plasmatic membrane (plasmalemma) of cells from the epidermis and root cortex it is reduced to nitrite (NO_2^-) in the cytosol and, immediately after, converted to ammonium (NH_4^+) in the plastid. Ammonium is then incorporated into amino acids by the enzymes glutamine synthetase and glutamate synthetase, forming glutamine, glutamate and other amino acids and their metabolites (Masclaux-Daubresse et al., 2010).

According to the analysis of variance of data of stem diameter of 'BRS Topázio' cotton at 30 DAS, there was a

Table 2. Summary of the analysis of variance for plant height (PH), stem diameter (SD) and leaf area (LA) of cotton plants irrigated with saline water under potassium and nitrate/ammonium fertilization at 30 and 130 days after sowing.

Sources of variation	DF	Mean square					
		PH		SD		LA	
		Days after sowing					
		30	130	30	130	30	130
Nitrate and ammonium (PNA)	4	60.03*	67.96*	0.99*	1.30*	159145.72*	342294.00*
Potassium doses(DK)	4	10.63^{ns}	14.25^{ns}	0.12^{ns}	0.06^{ns}	73960.75^{ns}	81437.14^{ns}
Interaction (PNA x DK)	16	21.93^{ns}	30.02^{ns}	0.47^{ns}	0.41^{ns}	67958.83^{ns}	75476.19^{ns}
Blocks	2	26.01^{ns}	27.13^{ns}	0.22^{ns}	0.60^{ns}	3076.76^{ns}	122738.63^{ns}
Residue	48	16.95	19.72	0.25	0.27	51163.93	90301.33
CV (%)		9.57	8.11	8.66	6.35	20.36	19.44

ns, **, * represent respectively, not significant, significant at 0.01 and 0.05 probability level.

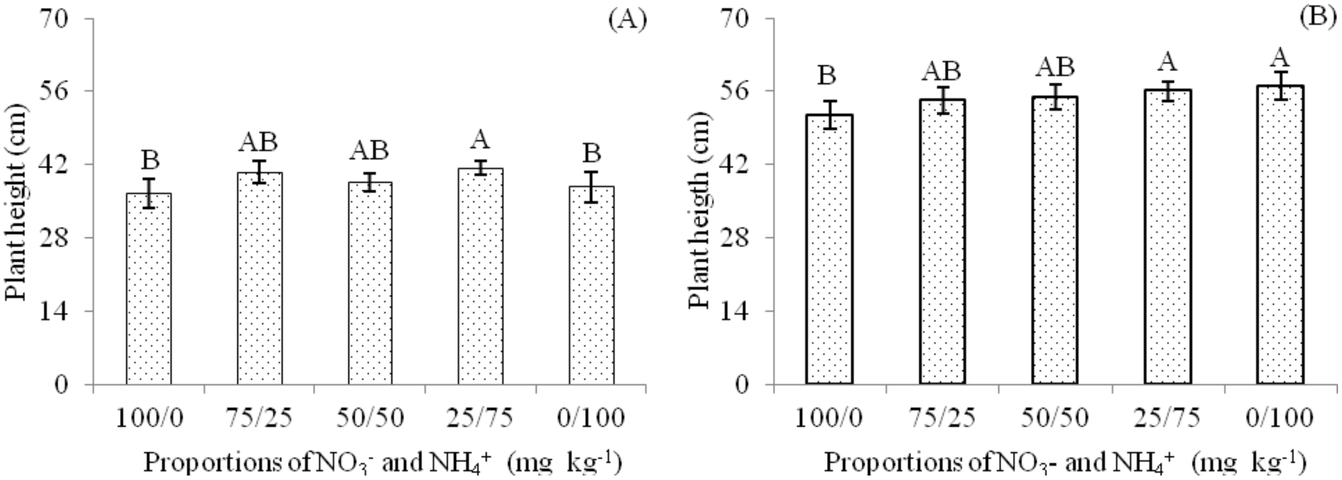

Figure 1. Plant height of 'BRS Topázio' cotton irrigated with saline water under different proportions of nitrate and ammonium fertilization at 30 (A) and 130 (B) days after sowing. Means followed by different letters indicate difference between treatments by Tukey test at $p<0.05$; Bars represent the mean standard error (n=3).

significant effect of the studied treatments (Table 2) and according to the means comparison test (Figure 2A), plants fertilized with 75/25 and 25/75 mg of NO_3^-/NH_4^+ were superior to plants fertilized with only nitrate (100/0 mg). For plants fertilized with variable proportions of NO_3^- and NH_4^+, the highest (6.17 mm) and the lowest (5.53 mm) values of stem diameter were observed for the applications of 25/75 and 100/0 mg of nitrate/ammonium, respectively.

On the other hand, at 130 DAS (Figure 2B), the highest stem diameter (8.80 mm) was obtained for the proportion of 25/75 mg of NO_3^-/NH_4, and there were significant differences in relation to NO_3^- and NH_4^+ proportions of 100/0, 75/25 and 50/50 (Figure 2B). The deleterious effect of excessive NO_3^- on plant growth has been observed in many species, among which cassava (Cruz et al., 2008) and rice (Holzschuh et al., 2009).

The proportions of NO_3^- and NH_4^+ significantly influenced the leaf area of 'BRS Topázio' cotton at 30 and 130 DAS (Figure 3A and 3B) and, according to the comparison of mean test, plants fertilized with 25/75 mg of NO_3^- and NH_4^+ showed the highest values of leaf area, statistically differing from the treatments 0/100 and 100/0 at 30 DAS and 0/100 at 130 DAS. However, comparing the absolute values obtained in the treatment 25/75 at 30 and 130 DAS (1198.08 and 1752.13 cm^2, respectively), an increase in leaf area of 242.14, 95.07, 119.48 and 239.84 cm^2 at 30 DAS and 423.34, 191.18, 234.69 and 181.98 cm^2 at 130 DAS was observed respectively for the treatments 100/0, 75/25, 50/50 and 0/100 of NO_3^- and NH_4^+.

According to Vale et al. (1998), the variation in cations/anions ratio (NO_3^- and NH_4^+: 25/75) shows the important role of N in the maintenance of ionic balance in plants. In the predominantly nitrate nutrition, the absorption of anions tends to supersede the absorption of

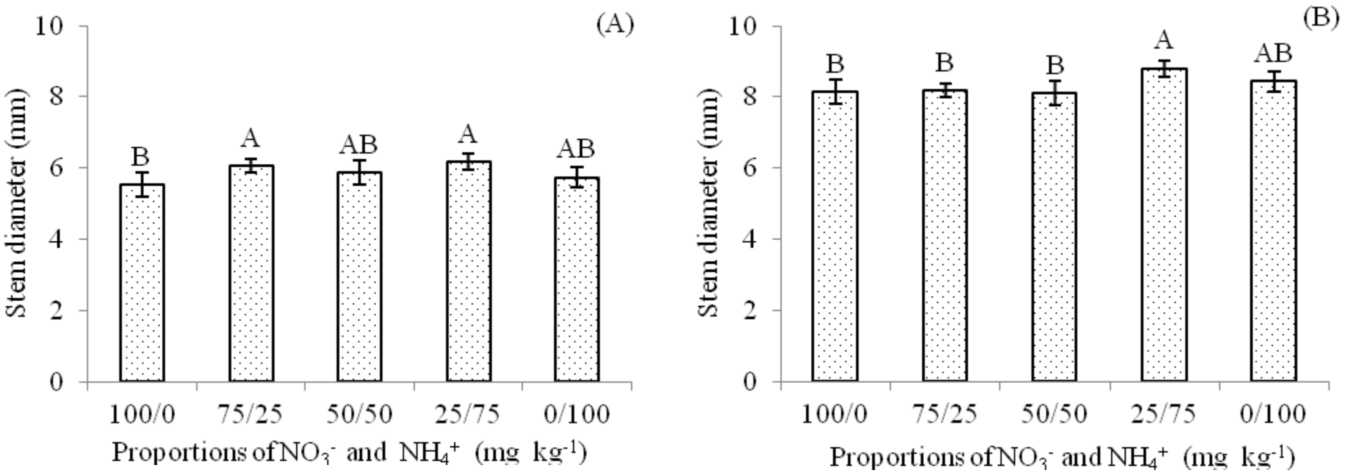

Figure 2. Stem diameter of 'BRS Topázio' cotton irrigated with saline water under different proportions of nitrate and ammonium fertilization at 30 (A) and 130 (B) days after sowing. Means followed by different letters indicate difference between treatments by Tukey test at $p<0.05$; Bars represent the mean standard error ($n=3$).

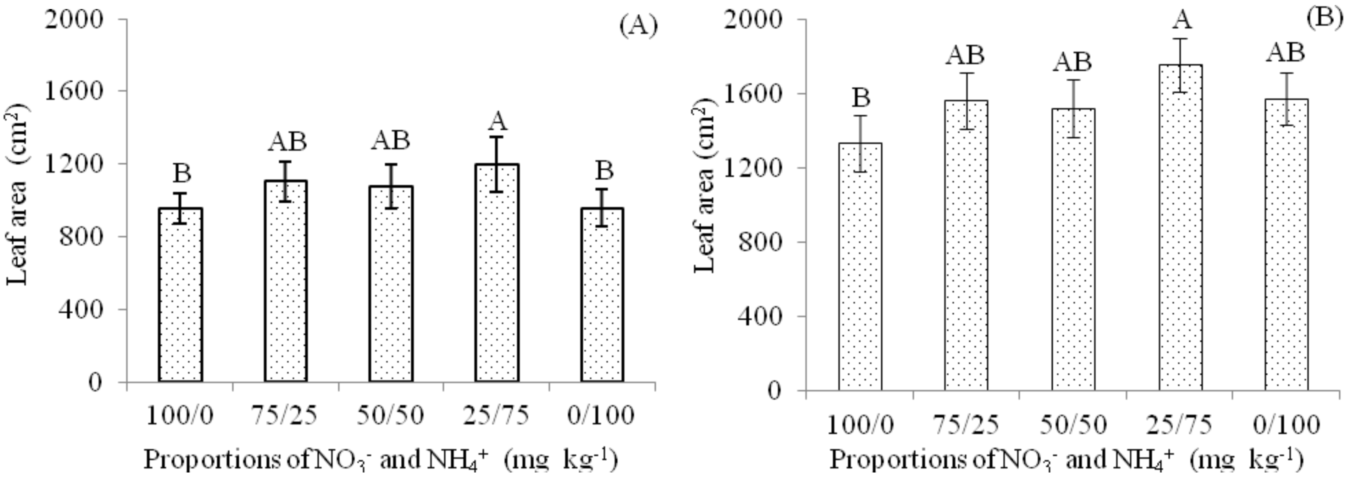

Figure 3. Leaf area of 'BRS Topázio' cotton irrigated with saline water under different proportions of nitrate and ammonium fertilization at 30 (A) and 130 (B) days after sowing. Means followed by different letters indicate difference between treatments by Tukey test at $p<0.05$; Bars represent the mean standard error ($n=3$).

cations, while in the predominantly ammoniacal nutrition, the total absorption of cations exceeds the absorption of anions and, in this case, the electroneutrality is maintained by the efflux of H^+ ions. Unlike the results obtained in this study, Cruz et al. (2006) evaluating cassava plants by applying different proportions of nitrate and ammonium (12:0, 9:3, 6:6, 3:9 and 0:12 mol m^{-3}) found that NH_4^+ is more harmful to leaf expansion as compared to the NO_3^-.

According to the summary of the analysis of variance (Table 3), there was a significant effect of the different proportions of nitrate and ammonium for cotton seed mass and mass of one boll. As to the factor K doses, there was a significant effect for cotton seed mass and number of bolls. On the other hand, the interaction between factors (PNA x DK) significantly influenced the number of days for flower bud opening and cottonseed mass.

The interaction between K doses and variable proportions of NO_3^-/NH_4^+ interfered with the number of days for flower bud opening only when plants were fertilized with 125% of recommended dose of K (Figure 4A), and significant differences occurred in the number of days for flower bud opening, with reductions of 7.60% for the proportion of 100/0 when compared with 75/25 mg of N (NO_3^-/NH_4^+). This increment in NFBO for plants subjected to 25/75 (Figure 4A), in comparison to the other studied treatments, is probably related to the fact

Table 3. Summary of the analysis of variance for number of days for flower bud opening (NFBO), number of bolls per plant (NBP), cottonseed mass (CSM) and mass of one boll (M1B) of cotton irrigated with saline water under potassium and different proportions of nitrate and ammonium fertilization.

Sources of variation	DF	Mean square			
		NFBO	CSM	NBP	M1B
Nitrate and ammonium (PNA)	4	3.16^{ns}	261.01**	1.75^{ns}	17.25*
Potassium doses (DK)	4	6.23^{ns}	87.00*	5.28*	1.39^{ns}
Linear regression	4	5.60^{ns}	4.95^{ns}	2.16^{ns}	0.76^{ns}
Quadratic regression	4	0.38^{ns}	22.36^{ns}	17.14^{ns}	1.01^{ns}
Interaction (PNA x DK)	16	5.73*	85.16**	2.30^{ns}	3.95^{ns}
Blocks	2	1.88^{ns}	54.52^{ns}	3.33^{ns}	3.76^{ns}
Residue	48	3.35	15.29	1.55	3.14
CV (%)		3.40	20.61	22.98	16.78

ns, **, * respectively, not significant, significant at 0.01 and 0.05 level of probability.

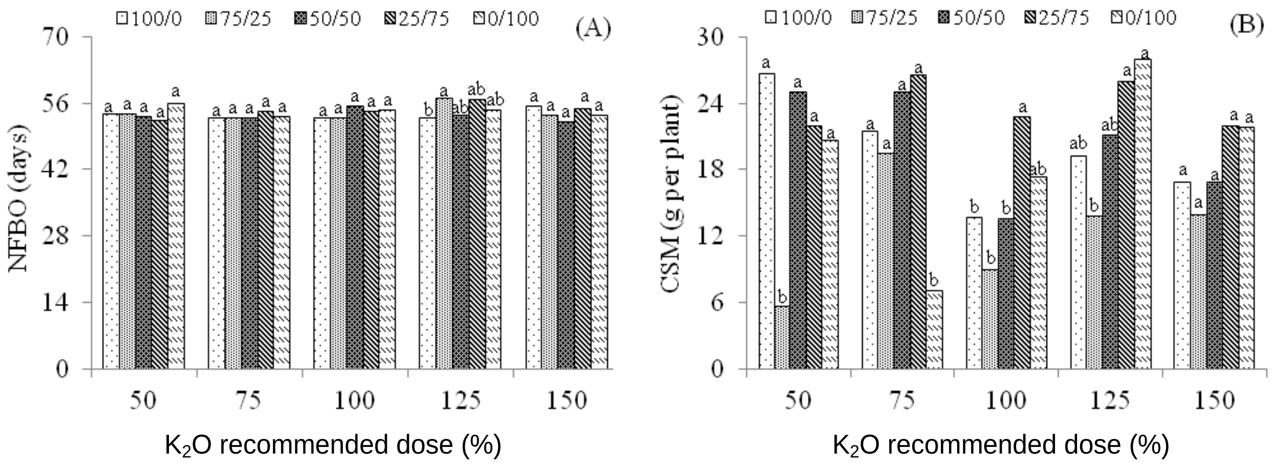

Figure 4. Number of days for flower bud opening-NFBO (A) and cottonseed mass-CSM (B) of 'BRS Topázio' cotton irrigated with saline water under different doses of K_2O and proportions of nitrate and ammonium fertilization. Means followed by different letters indicate difference between treatments by Tukey test, $p<0.05$.

that, in both evaluation periods (30 and 130 DAS), the carbohydrates produced by plants were primarily used for the growth of 'BRS Topázio' cotton, which is evidenced by the increment in the variables PH, SD and LA. This energy expenditure in the vegetative stage may have contributed to the delay in the number of days for flower bud opening (Helali et al., 2010).

Cotton seed mass was significantly influenced by the interaction between the factors K doses and proportions of NO_3^-/NH_4^+ (Table 3) and, according to the mean comparison test (Figure 4B), fertilization with 75/25 mg of NO_3^-/NH_4^+ promoted lower production in cottonseed mass (5.64 g plant^{-1}), when plants were subjected to 50% of the recommended dose of K (75 mg of K_2O), whereas, the proportions of 25/75 and 0/100 mg of NO_3^-/NH_4^+ when combined with doses of 75 and 125% (112.5 and 187.5 mg of K_2O), promoted higher cottonseed mass (26.53 and 27.94 g plant^{-1}, respectively).

The number of cotton bolls was significantly influenced ($p<0.05$) by the different K doses and, according to the regression equation (Figure 5A), the dose of 97% of the recommendation (145.5 mg of K_2O) promoted the maximum value for this variable (on average, 6.09 bolls plant^{-1}). In addition, the minimum value for NBP (on average, 4.71 bolls plant^{-1}) was observed in plants fertilized with the dose of 150% (225 mg of K_2O). The main hypothesis to explain the observed decrease in the number of cotton bolls in the highest K dose (150%) is based on competitive absorption, in which K is frequently absorbed by many species in amounts greater than the necessary in view of high concentration, inhibiting the absorption of Ca^{+2} and Mg^{+2} (Meurer, 2006). Besides this, the reduction in the number of cotton bolls is a response, for the most part, the osmotic effects, which restrict the absorption of water caused by the high concentration of soluble salts in the soil (especially KCl) as well as the

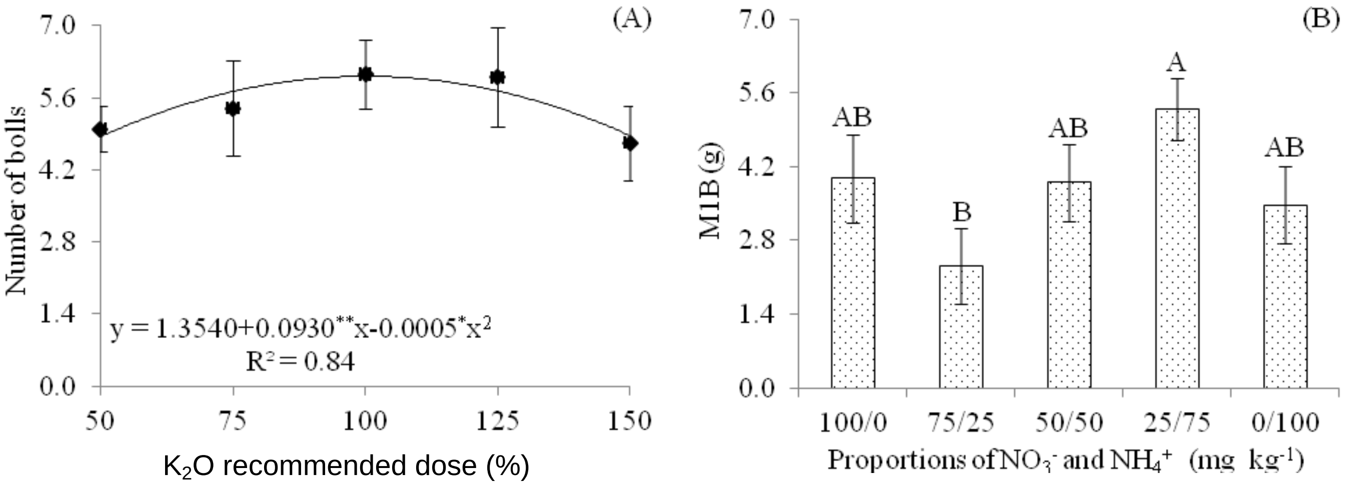

Figure 5. Number of bolls per plant-NBP as a function of the doses de K_2O (A) and mass of one boll-M1B as a function of the different proportions of nitrate and ammonium fertilization (B) of the 'BRS Topázio' cotton irrigated with saline water. Bars represent the mean standard error (n=3).

entry of ions in an amount enough to cause toxicity, causing imbalance in the absorption of nutrients, leading to widespread reduction in its growth, with serious losses in crop yields. Similar results were also reported by Andriolo et al. (2010), who evaluated the effects of K doses supplied through fertigation on growth, production and quality of strawberry fruits. These authors concluded that the increase in K concentration in the nutrient solution decreases growth, production and organoleptic quality of fruits. Lima (2014), evaluating the castor bean cultivar 'BRS Energia' as a function of salinity and the cationic nature of the irrigation water, observed reduction in the production components of plants subjected to irrigation with water of potassium composition.

The mean mass of one boll (Figure 5B) was significantly different ($p<0.05$) due to the application of different proportions of NO_3^-/NH_4^+. Plants subjected to 25/75 mg of nitrate and ammonium showed higher M1B (5.30 g), significantly differing from plants in the treatment with 75/25. On the other hand, M1B values of plants fertilized with 100/0, 50/50, 25/75 and 0/100 mg of NO_3^- and NH_4^+ did not differ statistically. These results reflect the tendency observed for cotton growth variables (PH, SD and LA) evaluated at 30 and 130 DAS, for which the proportion of 25/75 of NO_3^-/NH_4^+ promoted higher growth and, consequently, higher production, which is evidenced by the mass of one boll.

Conclusions

1. The supply of NO_3^- and NH_4^+ in the proportion of 25/75 mg of N kg^{-1}, respectively, promotes an increase in growth variables (plant height, stem diameter and leaf area) and in the mass of one boll of 'BRS Topázio' cotton;
2. The dose of 145.5 mg of K_2O (97% of the recommendation) promotes the formation of higher number of bolls per plant;
3. The application of potassium in the doses of 112.5 and 187.5 mg of K_2O (75 and 125% of the recommendation) combined with proportions of 25/75 and 0/100 mg of NO_3^-/NH_4^+ promotes an increment in cotton seed mass, and the dose of 187.5 mg of K_2O (125% of the recommendation) associated with 25/75 mg of NO_3^- and NH_4^+ increase the number of days for flower bud opening.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Effect of two dipping pretreatment on drying kinetics of golden berry (*Physalis peruviana* L.)

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Golden berry (*Physalis peruviana* L.) has scientifically proven medicinal properties. This research was aimed to investigate the effect of alkaline ethyl oleate and sucrose solution pretreatments as well as drying temperature on the drying kinetics of golden berry. Based on this, golden berries were dipped in alkaline ethyl oleate solution (2% ethyl oleate + 4% potassium carbonate) at 25°C or in osmotic solution (40% sucrose) at 60°C. After those pretreatments, air drying characteristics of golden berry (*P. peruviana* L.) were investigated at 70, 75, 80 and 85°C with 1.5 m/s air flow rate. Also, golden berries were dried without pretreatment and taken as a control. The experimental data was fitted to Page model. Two statistical tools were used to quantify the goodness of fitting: the determination of coefficient and the reduced chi-square. Obtained effective diffusivity for pretreatment of alkaline ethyl oleate and sucrose solution and untreated samples varied between 1.94 to $3.19 \cdot 10^{-9}$ m^2/s, 1.21 to $2.93 \cdot 10^{-9}$ and 1.66 to $2.67 \cdot 10^{-9}$ m^2/s respectively.

Key words: *Physalis perviana* L., alkaline ethyl oleato, osmotic dehydration, effective diffusivity, Page model

INTRODUCTION

Physalis peruviana, is known as “Güvey Feneri” in Turkey, “Uvilla” in Ecuador, “Topotopo” in Venezuela and “Golden Berry” in English speaking countries. Generally, the fruit of *P. peruviana* is consumed fresh which provides an acid-sweet balance of fruit and vegetable salads. Also, the whole fruit can be dried and it can be consumed as a very nice raisin (Puente et al., 2011). Golden berry (*P. peruviana* L.) has scientifically proven medicinal properties such as anticancer, antimicrobial and antipyretic effects (Franco et al., 2007; Puente et al., 2011). The major objective in drying agricultural products is the reduction of the moisture content to a level which allows safe storage over an extended period (Doymaz, 2004). Golden berry is a highly perishable fruit that is a strong limiting factor on its commercialization. So that studies on drying of golden berry is thought as necessary and fulfill the requirement of industry.

Several fruits and vegetables, such as grapes, plums, apricots, peppers, tomatoes and physalis are covered naturally with a thin layer of wax. This outer layer offers benefits such as protection to the fruit or vegetable from environmental and external factors. The wax layer also

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Table 1. Color, size, weight and dry matter of fresh golden berry.

Characters	Results
Color values (L, a, b)	61.51±1.69, 11.58±2.53, 52.82±1.92
Size (diameter)	2.5 ± 0.1 cm
Weight	7.7 ± 0.3 g
Dry matter	18.43±0.5%

affects the flow of moisture from inside of the fruit to its surface which is a crucial process in drying (Sagar and Suresh, 2010). Due to that, chemical dipping pre-treatments such as methyl ethyl ester emulsion or alkaline pretreatment have been used prior to the drying process. Prior to drying process, chemical dipping such as methyl and ethyl ester emulsions or alkaline pretreatment in aqueous solutions of sodium hydroxide, sodium chloride, potassium carbonate and calcium chloride have been used to overcome the wax barrier on fruits or vegetables.

Dipping waxy fruits for several seconds in solution of ethyl oleate or other suitable compound greatly reduces drying time. The effects of dipping solutions on various fruits and vegetables during drying are reported in literature (Riva and Peri, 1986; Doymaz and Pala, 2002). Before drying, fruits or vegetables are pretreated in various solutions such as calcium chloride (Al-Harahsheh et al., 2009), sodium chloride (McMinn and Magee,1999; Sacilik et al., 2006), and sodium chloride-sucrose (Azoubel et al., 2004) and then can be dried in different shapes such as halves, slices and quarters (Souza et al., 2007; Cernisev, 2010). However, no reports have been found detailing the effects of alkaline ethyl oleate solution or sucrose solution pretreatment on drying of golden berry in the literature. This study was aimed to investigate the effect of alkaline ethyl oleate and sucrose solutions on golden berry drying period, to calculate effective moisture diffusivity and to fit the experimental data with Page model.

MATERIALS AND METHODS

Mature golden berries were harvested in observation garden of Ataturk Central Horticultural Research Institute (Yalova, Turkey). Color, size, weight and dry matter of fresh golden berry were given in Table 1.

Dipping pretreatment

Golden berries were washed in fresh running water and divided in three sample groups. One sample group was dipped in alkaline ethyl oleate (AEO) solution which contained 2% ethyl oleate and 4% potassium carbonate at 25°C for 1 min. Other one sample group was dipped in 40% sucrose solution (SC) at 60°C. The AEO or SC volume to golden berry weight ratio were kept ratio 4:1. No pretreatment (NP) was applied to one sample group and dried as a control group.

Drying

Drying experiments were performed in a laboratory scale hot-air dryer which was illustrated in Figure 1. This dryer was installed in the Food Technology Department of Ataturk Central Horticultural Research Institute; desired experiment conditions inside the dryer were obtained for at least 1 h prior to each run.

Pretreated and non pretreated sample groups were spread on a perforated tray. Drying runs of all sample groups were conducted at four temperatures (70, 75, 80 and 85°C) with fixed 1.5 m/s air flow both ascending and descending. Moisture loss was recorded automatically during drying by means of a digital balance (Nuve, model FN500S) with an accuracy of ±0.01 g. The drying was carried out to final moisture content reduced to 3%. Drying process was applied in triplicate.

Mathematical modeling of drying curves

The moisture ratio (*MR*) of samples was calculated using the following equation:

$$MR = \frac{M_t - M_e}{M_0 - M_e} \qquad (1)$$

where M_t is the water content at any time (kg water/kg dry solid), M_0 is the initial water content (kg water/kg dry solid), and M_e is equilibrium water content of sample (kg water/kg dry solid). The values of M_e are relatively small compared to M_t or M_0, thus the error involved in the simplification is negligible (Diamante and Munro, 1993). In this study, M_e was accepted as zero.

Obtained drying curves of the golden berries were fitted with Page model. This model is widely used to describe the drying characteristics of various vegetables and fruits such as kiwi fruit**,** pear, mango, potato, eggplant and cherry tomato (Diamante and Munro, 1993; Ertekin and Yaldiz, 2004; Simal et al., 2005; Goyal et al., 2006). The model is written as follows:

$$MR = exp(-kt^n) \qquad (2)$$

where *k* and *n* are drying constant.

Calculation of effective diffusivity and activation energy

The effective diffusivity (D_{eff}) is also typically calculated according to Sobukola et al. (2007) by using the slope of Equation (3), namely, when natural ln (*MR*) versus time was plotted, a straight line with a slope k_0 was obtained:

$$MR = \frac{8}{\pi^2} exp\left(\frac{\pi^2 D_{eff}\ t}{4\ L^2}\right) \qquad (3)$$

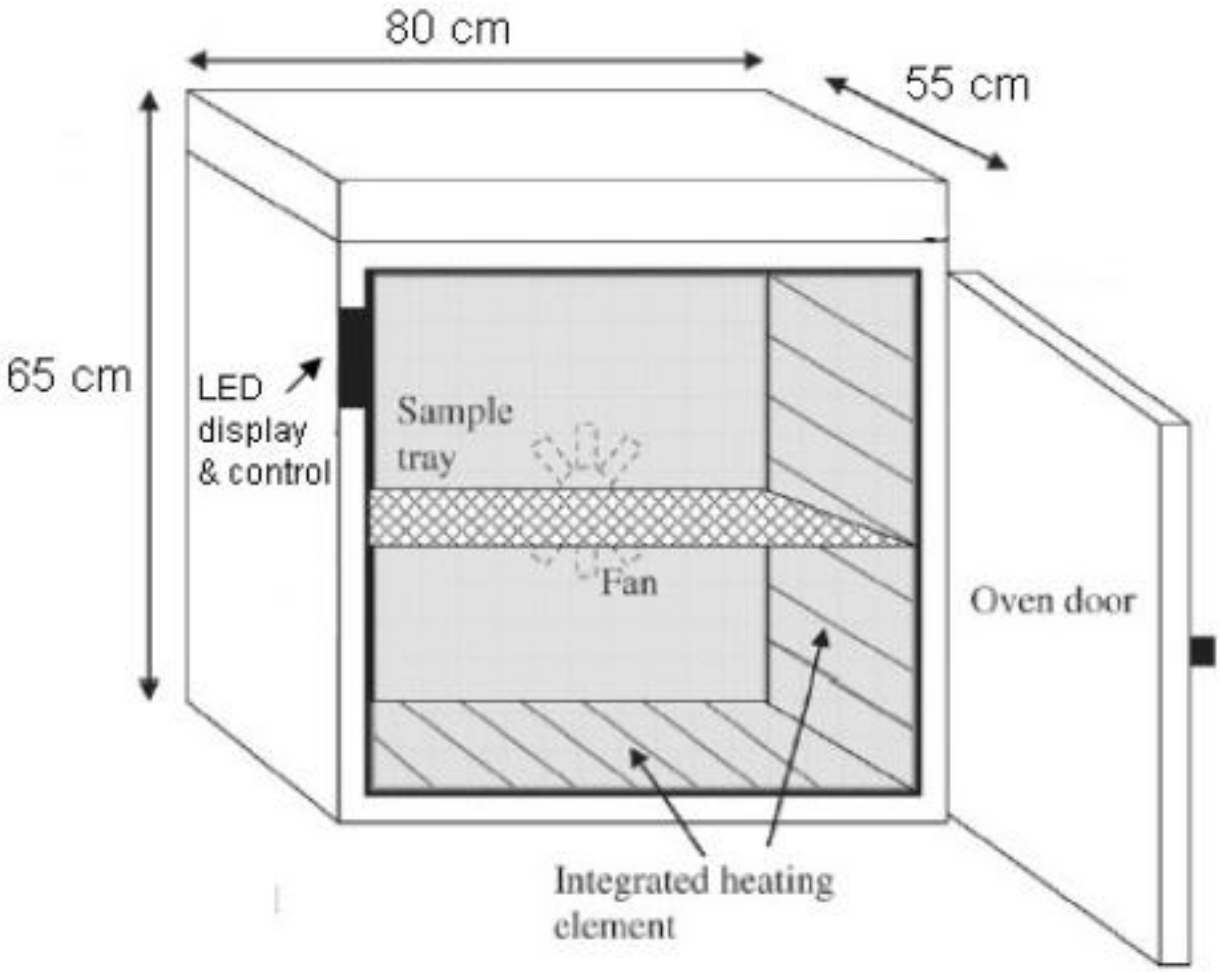

Figure 1. Schematic view of the dryer.

$$k_0 = \frac{\pi^2 D_{eff}}{4 L^2} \quad (4)$$

t: drying time (h)
L = average diameter of golden berry (m).

Analysis of drying process

Page moisture ratio model was fitted to the drying data and the model parameters determined using non-linear regression analysis. The terms used to evaluate goodness of fit of the tested models to the experimental data were the coefficient of determination (R^2) and the reduced chi-square (X^2) between the experimental and predicted moisture ratio values. The reduced chi-square (X^2) could be calculated as following:

$$X^2 = \frac{\sum_{i=1}^{N} (MR_{exp,i} - MR_{pre,i})^2}{N-z} \quad (5)$$

where $MR_{exp,i}$ and $MR_{pre,i}$ were experimental and predicted moisture ratios, respectively, *N* was number of observations, and *z* was number of drying constants (Sarsavadia et al., 1999).

RESULTS AND DISCUSSION

Effect of pretreatments on drying time

Effect of the pretreatments and drying temperatures on drying time of golden berry were given in Table 2. Moisture content of golden berry is reduced to 15% and after that 3% for evaluating and fitting of drying model. Increase in drying temperature was determined to be caused dramatic reduction on drying time. Similar results were given in literature for golden berry (Valdenegro et al., 2013), tomato (Cernisev, 2010) and grape (Doymaz, 2006). Drying times to reduce the final moisture content to 15% was investigated in the order NP>SC>AEO at all temperature levels in this study. Thus, it can be seen that the pretreatment using dipping immersion in AEO was more effective than sucrose solution in to reduce the drying time. Fresh and dried golden berry samples were given in Figure 2. The effects of the pretreatments on the moisture ratio of the golden berry over drying time were shown in Figures 3 to 6. From these figures, pretreatment

Table 2. Determined drying time of golden berry samples to reduce their final moisture content to 15% and 0%.

Temperature (°C)	Drying time for 15% final moisture (min)			Drying time for 2% final moisture (min)		
	AEO	**SC**	**NP**	**AEO**	**SC**	**NP**
70	550±7	550±7	600±9	990±12	1050±14	1140±22
75	570±7	570±7	590±9	900±8	930±8	1020±18
80	460±4	460±5	540±6	780±5	850±6	870±13
85	370±4	370±5	410±5	660±7	690±5	720±8

(a) (b)

Figure 2. Fresh (a) and dried (b) (dried at 70°C to 3% dray matter) golden berry samples.

Table 3. Decreasing ratio of drying time of AEO and SC pretreated samples in comparison with control during drying to15% water content.

Drying temperature (°C)	Decreasing in drying time (%)	
	AEO pretreated sample	**SC pretreated sample**
70	15.69±1.23^{d}	5.86±0.65^{c}
75	20.71±1.28^{b}	13.57±1.14^{a}
80	21.32±1.12^{a}	12.50±1.12^{b}
85	19.10±1.74^{c}	12.36±0.95^{b}

Different letters in same column indicate significant differences at $P < 0.05$.

solution was detected as an important factor for the golden berry drying because it affected the drying time.

Hence, AEO and SC could decrease the drying time more than untreated samples to reduce the moisture content to 15% for all temperatures. Decreasing ratio of drying time of AEO and SC pretreated samples in comparison with control was given in Table 3. It can be seen that the increase in temperature used promoted a considerably reduction in drying time (Table 3).

Similar results were found for alkaline ethyl oleate in the air drying of grapes (Doymaz, 2006), red pepper (Doymaz and Pala, 2002), apricot (Doymaz, 2004). In this research osmotic pretreatment reduced the drying time less than results of some research such as golden berry (Castro et al., 2008), acerola fruit (Agnelli et al., 2005), apple (Sereno et al., 2001), melon (Telas et al., 2006) and tomato (Souza et al., 2007). Expectedly, during the initial stages of drying there was a rapid moisture removal from the product which later decreased with increase in drying time. Remaining quantity of water is bounded more strongly while its quantity decreased so that drying rates decreased when moisture content decreased. Under the monolayer moisture level, water is firmly bound to the solutes of the golden berry so that in this study water was hardly removed when remaining water were decreased. From Figures 3 to 6, it can be seen that the moisture ratio decreases continually with drying time. As expected, the drying air temperatures had statistically significant effect on the drying moisture content of golden berry.

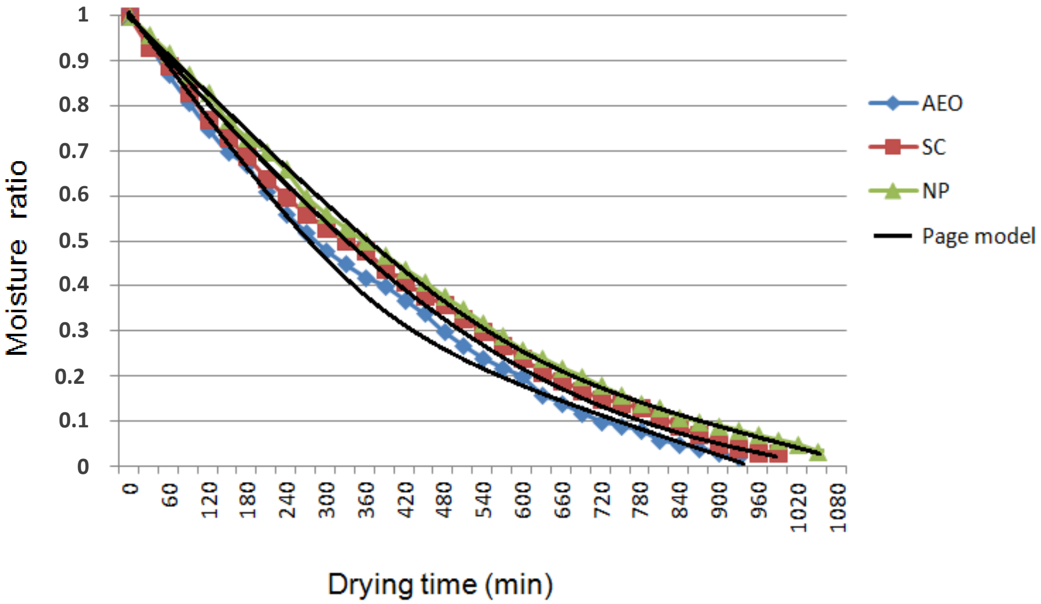

Figure 3. Variation of experimental and predicted moisture ratio by the Page model with drying time at 70°C for pre-treated and untreated golden berry.

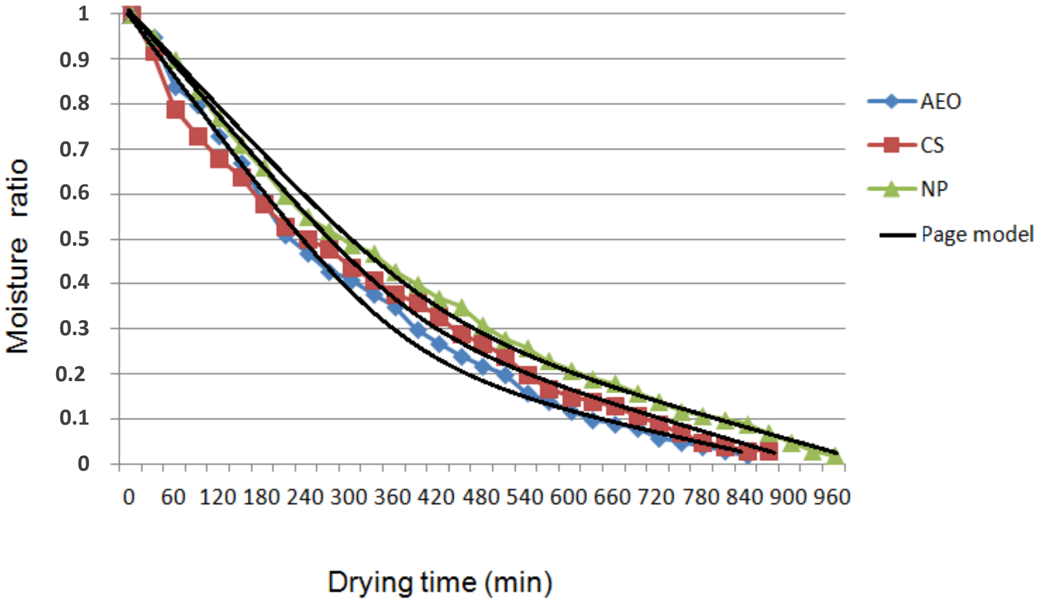

Figure 4. Variation of experimental and predicted moisture ratio by the Page model with drying time at 75°C for pre-treated and untreated golden berry.

The temperature influence was highest at 85°C air temperature, as expected. There was no constant rate drying period in these curves, all drying processes occurred in falling rate-drying period. During the falling drying rate period, the drying process of golden berry was mainly controlled by diffusion mechanisms. Similar results have been reported in the literature for various fruits and vegetables (Diamante and Munro, 1993; Azoubel et al., 2004; Lahsasni et al., 2004; Simal et al., 2005; Akanbi et al., 2006; Goyal et al., 2006).

Modeling of drying kinetics

The obtained drying data were fitted by Page model. The results of statistical analysis for Page model were shown

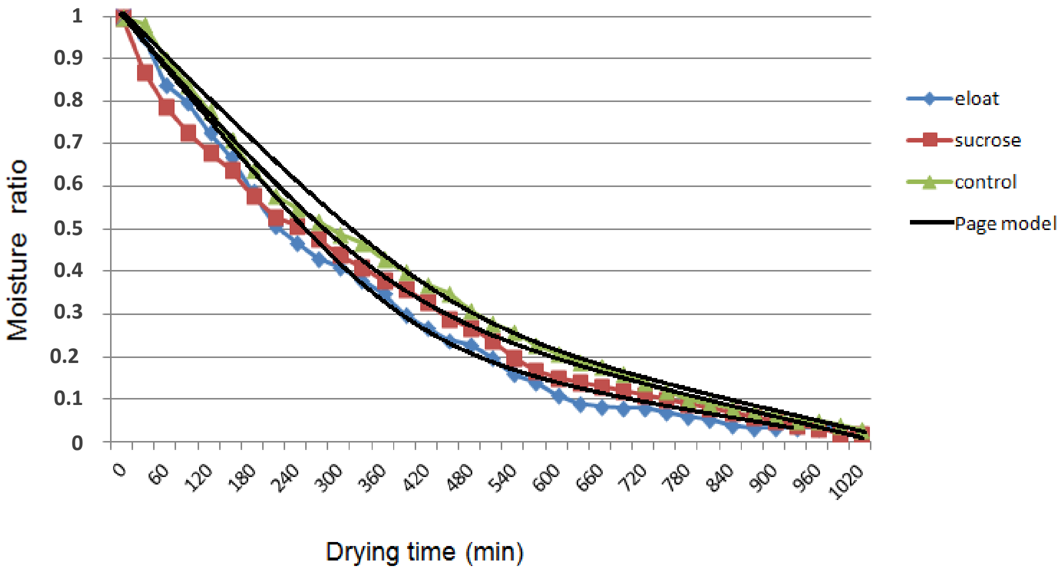

Figure 5. Variation of experimental and predicted moisture ratio by the Page model with drying time at 80°C for pre-treated and untreated golden berry.

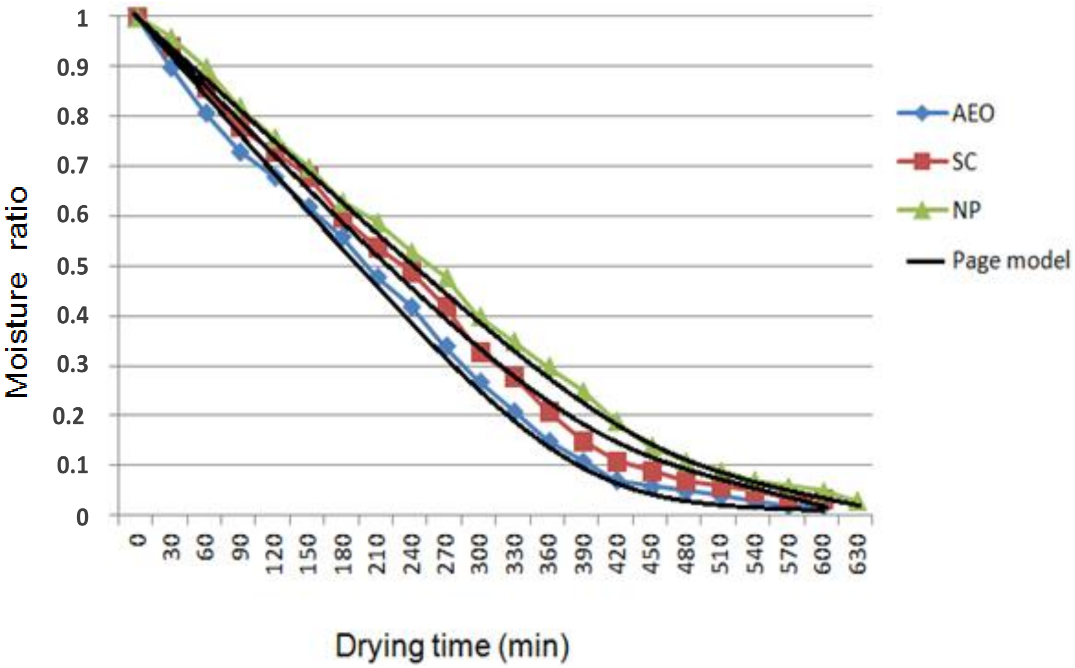

Figure 6. Variation of experimental and predicted moisture ratio by the Page model with drying time at 85°C for pre-treated and untreated golden berry.

in Table 4. Acceptable R^2 of greater than 0.98 were obtained for model fitted to all drying tests. The R^2 ranged from 0.9917 to 0.9956 in Page model. The X^2 value varied from 0.00014 to 0.00068. Similar results about Page model have been reported by Sacilik et al. (2006). Figures 3 to 6 showed the variations of experimental and predicted moisture ratios by the Page model withdrying time. According to these figures, Page model showed good agreement with the experimental data obtained from the drying experiments. Similar results were reported in the literature for some vegetables and fruits (Doymaz and Pala, 2002; Azoubel et al., 2004; Lahsasni

Table 4. Curve fitting criteria for the thin layer drying models for drying of tomatoes.

Code	T°C	Page model constants		R^2	X^2
		k	n		
AEO	70	0.0004	1.094	0.9931	0.00016
	75	0.0005	1.0644	0.9956	0.00043
	80	0.0005	1.1208	0.9925	0.00052
	85	0.0004	1.1163	0.9917	0.00068
SC	70	0.0003	1.0880	0.9932	0.00014
	75	0.0006	1.0620	0.9942	0.00037
	80	0.0004	1.1074	0.9950	0.00056
	85	0.0005	1.1109	0.9924	0.00053
NP	70	0.0006	1.083	0.9913	0.00014
	75	0.0004	1.0625	0.9948	0.00026
	80	0.0006	1.1126	0.9939	0.00039
	85	0.0005	1.1107	0.9942	0.00046

Table 5. Values of effective diffusivity obtained for golden berry samples at different temperatures.

Code	Temperature (°C)	D_{eff} (m^2/s)
AEO	70	1.94×10^{-9}
	75	2.02×10^{-9}
	80	2.70×10^{-9}
	85	3.19×10^{-9}
SC	70	1.71×10^{-9}
	75	1.81×10^{-9}
	80	2.69×10^{-9}
	85	2.93×10^{-9}
NP	70	1.73×10^{-9}
	75	1.66×10^{-9}
	80	1.90×10^{-9}
	85	2.67×10^{-9}

et al., 2004; Simal et al., 2005; Al-Harahsheh et al., 2009).

Calculation of effective diffusivity

Modeling of drying kinetics, as well as acquiring data on desorption isotherm or diffusion coefficient, is needed by the industry to manage efficiently dehydration techniques and avoid energy misuse (Vega-Gálvez et al., 2014). The calculated values of D_{eff} for different temperatures were presented in Table 5. The effective diffusivity values of dried samples at 70 to 85°C were varied in the range of 1.94 to 3.19×10^{-9} m^2/s for AEO pretreated samples, 1.71 to 2.93×10^{-9} m^2/s for SC pretreated samples and 1.66 to 2.67×10^{-9} m^2/s for untreated samples (control group).

Determined effective moisture diffusivity were similar with found by Vega-Gálvez et al. (2014) and higher than that found by Vásquez-Parra et al. (2013). The obtained values were in the suitable range for other similar sized products reported in the literature such as grape (Karathanos and Belessiotis, 1997). It can be seen that the values of D_{eff} increased greatly with increasing temperature. Drying at 85°C gave the highest D_{eff} values. D_{eff} values for golden berries were different to those estimated by different authors for other vegetables: 3.72 to 12.27×10^{-9} m^2/s for tomatoes dried from 45 to 75°C (Akanbi et al., 2006); 2.02×10^{-9} m^2/s for hot air drying of paprika at 60°C (Ramesh et al., 2001); 0.87 to 1.0×10^{-9} m^2/s for cherry tomato dried from 40 to 60°C (Varadharaju et al., 2001); 1.79 to 4.45×10^{-9} m^2/s for apple slices at 60°C (Velic et al., 2004); 2 to 4.2×10^{-10} m^2/s for garlic slices dried from 50 to 90°C (Madamba et al., 1996). Some of these values were lower than and some of these similar with estimated D_{eff} values of golden berry in this research. In these dyring kinetic studies of foods D_{eff} values were determined at different levels because of different drying parameters such as temperature and relative humidity, dried material and used drying equipments.

Alkaline ethyl oleate and sucrose solutions were used as pretreatment for golden berry drying process affected strongly the characteristics of the dried product. Pretreated golden berry with alkaline ethyl oleate solution dried faster than untreated samples. Drying curves of golden berries showed a falling rate drying period; did not showing a constant rate drying period. Page exponential model was considered as appropriate for explaining the drying features of golden berries.

Golden berry has a small size like chery tomato so that it has higher diffusion coefficient than other fruits and vegetables which have bigger size. In addition, high core content and different texture structure of golden berry may cause increase on diffusion coefficient when compared with other fruits and vegetables during same drying conditions such as temperature and relative humidity. AEO and SC pretreatment could reduce the drying time of golden berry so that energy could be saved.

Conflict of Interests

Authors disclose that there is no financial/relevant conflict of interests.

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Full Length Research Paper

Influence of the land use on the water quality in the São João and Iguaçu Rivers, state of Paraná, Brazil: assessment of the importance of the riparian zone

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Further objectives were to determine the different patterns of land use and occupation within the microbasin formed by these rivers, and to assess the influence of the quantity of riparian vegetation on the presser/vation of the aquatic ecosystems. The studied basin extends over a total area of 77.75 km^2, of which 92.98% comprised agriculture (44.11%) and native forest (48.87%). The drainage area of the sampling site P1 was 87.57% covered by crops, and so was 22.30% of the area of the sampling site P4. However, the application of the Tukey test (p < 0.05) to the data for thermotolerant coliforms (also known as faecal coliforms - FC) led to the inference of similarity (p<0.05) between the sampling sites P1 and P4. This indicates that the agriculture impact is probably mitigated by the area of native forest around the source of the spring that feeds P1. From the sampling site P2 to P3, the drainage area showed an improvement in the preservation of natural areas, with the area covered by crops decreasing to 44.41% and an increase of 47.93% in the land covered by native forest. Sampling sites P2 and P3 also showed significant similarity between their annual averages for FC, displaying a reduction in the concentration of these microorganisms from 2.15 to 1.61 (× 10^3 MPN/100 mL), which again provided evidence of the positive influence of the recovery of plant cover within the drainage area. Recognising that the other sampling sites with very similar land use characteristics did not display significant alterations in the water quality, the influence of the vegetation and its role as a buffer were made evident, since for these sampling sites most water sources are not bereft of surrounding vegetation. In this way, the lack of correlation (Pearson and Spearman) between the seasonal variations for the assessed water quality parameters allowed us to provide evidence for the positive effect of riparian vegetation in the maintenance of the integrity of aquatic ecosystems and the preservation of the water quality. The water quality comprises physical, chemical, and microbiological parameters related to the different land uses within the river basin.

Key words: Monitoring, total and thermotolerant coliforms, biological indicators, human impact, water quality, multifactorial analysis.

INTRODUCTION

Hydrographic basins where farming takes place have higher concentrations of sediments and nutrients in their flowing waters in comparison to hydrographic basins protected by native vegetation (Allan, 2004). Over time, the various impacts of agriculture and grazing cause a continuous decrease in plant cover around aquatic environments. In addition, the use of land for farming and pastures reduce surface roughness, infiltration, and evapotranspiration (Hoffman and Ries, 1999), which in turn decrease plant root lengths rendering the surface more susceptible to erosion (Beeson and Doyle, 1995).

The integrity of riparian forests lessens the removal of adjacent soil, the loss of aquatic habitats, and the decline of biological diversity by mitigating the effects of surface runoff (Berkman and Rabeni, 1987). When these functions are jeopardised by alterations in the composition of the riparian zone, this zone becomes unstable and may no longer be able to ameliorate changes in physical, chemical (Monaghan and Smith, 2012), and biological (coliforms) parameters of the water resources, which result from surface runoff caused by rainfall (Hong et al., 2010).

Under Article 2 of the Brazilian Forestry Code (Law nº 4.771/65), riparian forests are categorised as Areas for Permanent Preservation (APP) (BRASIL, 1965). Improving the quality of the water in rivers is seen as a priority linked to the width of riparian forests, which is in turn related to the width of watercourses (BRASIL, 1965). In the case of small water basins, the extent and condition of the riparian vegetation can be used as hydrological indicators of the sustainability of human activities that are associated with different land uses (Lima and Zákia, 1998).

One means to assess the human interference through land use is monitoring local water resources (Coradi et al., 2009). The Brazilian National Environment Council (Conselho Nacional do Meio Ambiente, CONAMA), through resolution # 357/05, has established a set of parameters for the monitoring of water quality, corresponding to a number of physical, chemical and biological (total and thermotolerant coliforms) parameters (BRASIL, 2008).

The relations between riparian vegetation, land use, and water quality are not spatially stationary, but depend upon the individual characteristics of each hydrographic basin, for the physical features of the environment, economic activities, and pollution sources will not be constant in space (Tu, 2013).

Since around the analysed rivers the riparian forests are intact, our expectations were the following: (1) No significant variations between the seasons in the concentrations of total coliforms (TC) and thermotolerant coliforms (FC); (2) The type of land use in surrounding areas does not affect water quality in time, and (3) The existence of a direct relationship between the preservation of the riparian vegetation and water quality of the corresponding aquatic environments, regardless of the type of land use. Based upon this perspective, the present study had the following objectives: to determine the different types of land use and occupation for a neotropical river microbasin, to assess the pattern of variations in space and time for the water quality parameters of the rivers of such a basin, and to analyse the influence of riparian vegetation on the integrity of the aquatic ecosystems as a function of land use.

MATERIALS AND METHODS

Study area and sampling sites (collection points)

The present study was conducted on the São João River (SJ) and the Iguaçu River (IG), which belong to the hydrographic basin of the Iguaçu River, in the state of Paraná, Brazil. The source of the São João River is located in a rural area, and it drains into the right margin of the Iguaçu River. The Iguaçu River has its source within the city of Curitiba, the capital of the state of Paraná, and discharges into the west of the state, passing through the Iguaçu National Park, before reaching the Paraná River at Foz do Iguaçu.

The courses of both rivers are partially within the Federal Reserve (Unidade de Conservação Federal) known as Iguaçu National Park (ParNaIguaçu - 25° 05’ to 25° 41’ S and 53° 40’ to 54° 38’ W), and with regard to structure are considered typical aquatic ecosystems within this biome (IBAMA, 1999). The sampling sites were labelled P1 to P6; their locations were selected to reflect different land uses and interaction with the reserve. Sampling sites (collection points) P1 to P4 were along São João River, whereas P5 and P6 were located on the Iguaçu River (Figure 1).

Land use determination

A map of land use and occupation was constructed by visual classification of panchromatic images acquired from the updated database available by the Company Environmental Systems Research Institute (ESRI), making use of a geographic information system (GIS), as implemented in the software ArcGIS 10 (ESRI, 2010). Five classes of land use and occupation were defined: Native forest, pasture and reservoirs, agriculture, residential area, and areas of afforestation by exotic species.

Parameters monitored and analytical methods

A total of 72 water samples were assessed each month, from July 2011 to June 2012, by determination of a set of 22 variables in triplicate. Thermotolerant coliforms (FC) and total coliforms (TC) were determined by the Most Probable Number (MPN) technique as described in the manual of *Standard Methods* (APHA, 2005) and compared against the parameters specified by order # 2914 issued by the Ministry of Health (BRASIL, 2011a) and CONAMA resolution # 357/05 (BRASIL, 2008). Physical and chemical analyses of the water to yield the following: pH, temperature (T), turbidity (TUR), salinity (Sa), electrical conductivity (EC), dissolved oxygen (DO), total dissolved solids (TDS), and flow (Q) were performed *in loco*

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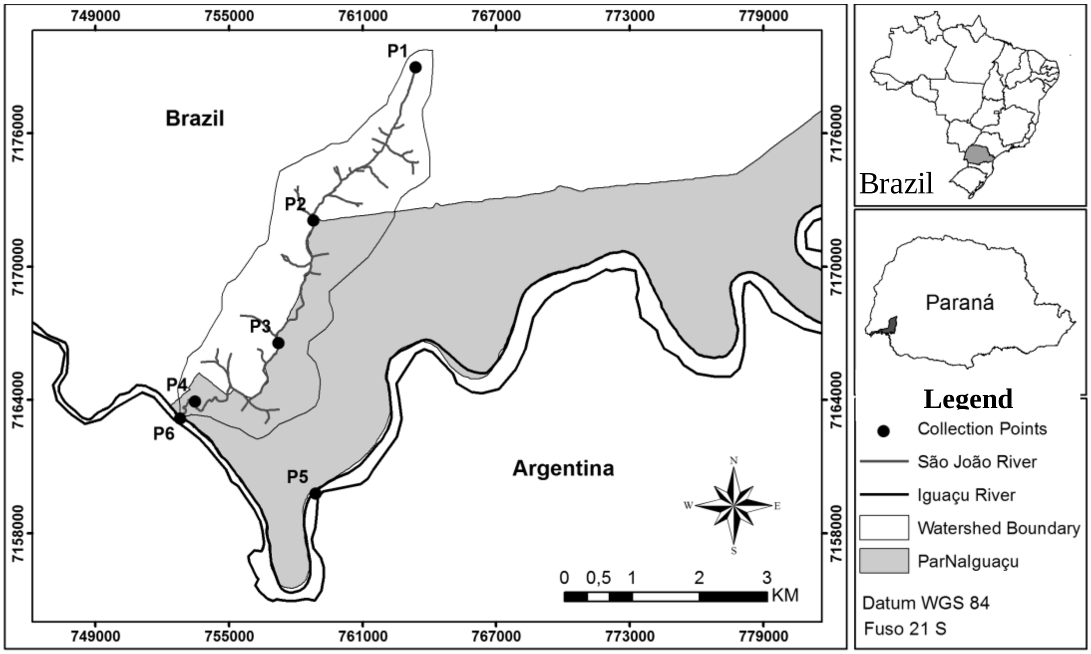

Figure 1. Location of the study area and collection points.

with a multiparameter instrument (Horiba® model CEL U50).

Biochemical oxygen demand (BOD - 5 days at 20°C), nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), ammonium (NH_4^+), total nitrogen (TN), phosphate (PO_4^{3-}), orthophosphate ($(PO_4^{3-})_2$), and total phosphorus (TP) were quantified according to the manual of *Standard Methods* (APHA, 2005) in the laboratories of the Iguaçu National Park (AquaIguaçu). Daily, weekly, and monthly rainfall, together with the volumetric flow rates of the Iguaçu and São João Rivers, were provided by the Instituto das Águas do Paraná (ÁGUAS PARANÁ, 2012).

Statistical analyses of the data

Analysis of variance was used for the main effects (One-way ANOVA), followed by comparison of means using the Tukey test ($p < 0.05$) so that the results could be compared against parameters set by legislation and to identify significant differences between the variables. The scores along the first three axes from the principal component analysis (PCA) were submitted to Two-way ANOVA (Main effects) using month and sampling sites as independent variables.

A multivariate approach was used to identify associations between the physical and chemical parameters: Principal Components Analysis (PCA), in which the determination as to the principal components to retain for data interpretation was set by the Kaiser-Gutman criterion (Jackson, 1993). The data were transformed to $\log_{10}(x+1)$ so that the frequency distributions approximated the normality condition.

In the assessment of the effects of rainfall (daily, weekly, and monthly) on the physical, chemical, and biological parameters, the scores along the retained axes were correlated to their abundances through Pearson and Spearman correlation tests. For both tests, correlations with $p < 0.05$ were considered significant.

For the purposes of determining the degree of similarity between sampling sites as revealed by biotic and abiotic parameters, a clustering analysis (CLUSTER) was performed using the Euclidian distances and UPGMA (Unweighted Pair Group Method with Arithmetic Average) as the joining method (McCune and Grace, 2002). The assessment of the similarities between the collection points was assessed using the ANOSIM (analysis not Parametric Similarity), assuming 9999 permutations and a 0.05 significance level.

The analyses were performed using the software packages PC-ORD 4.0® (McCune and Mefford, 1999), Statistica 7.0® (Statsoft, 2005), Sisvar® (Ferreira, 2007) and Bioestat® (Ayres et al., 2007).

RESULTS AND DISCUSSION

Land use and occupation

The values and the percentages of the classes that differentiate the main forms of land use and occupation within the São João River Microbasin, together with values for the areas of the basin that drain to each sampling site, are presented in Table 1.

The São João River basin is within the Iguaçu River Basin. The data characterising the land use for the areas that drain to sampling sites P5 and P6 (Iguaçu River) are not presented in Table 1, because these areas lie completely within the Iguaçu National Park, where the only permitted land use is native vegetation.

The total area of the São João River Basin is approximately 77.75 km^2 (Figure 2a); 92.98% of this area belongs to the classes of agriculture (44.11%) and native forest (48.87%). The area of the São João River Basin whose quality was monitored through P1 (springs located inside a private reserve, Reserva Particular de Patrimônio Natural de Santa Maria) was influenced primarily by

Table 1. Land use characterization for the microbasin of the São João River and the sampling sites.

Land use	Microbasin		P1		P2		P3		P4	
	Km²	(%)	Km²	(%)	Km²	(%)	Km²	(%)	Km²	(%)
Agriculture	34.30	44.11	0.53	87.57	19.71	76.28	8.69	31.87	5.35	22.30
Native forest	38.00	48.87	0.07	12.42	3.81	14.76	17.10	62.69	16.98	70.76
Pasture and reservoirs	3.75	4.82	0.00	0.00	1.83	7.10	0.62	2.29	1.28	5.37
Area of afforestation by exotic species	0.72	0.93	0.00	0.00	0.00	0.00	0.72	2.64	0.00	0.00
Residential area	0.98	1.27	0.00	0.00	0.47	1.84	0.13	0.49	0.37	1.56
Total area	77.75	100	0.60	100	25.84	100	27.28	100	24.00	100

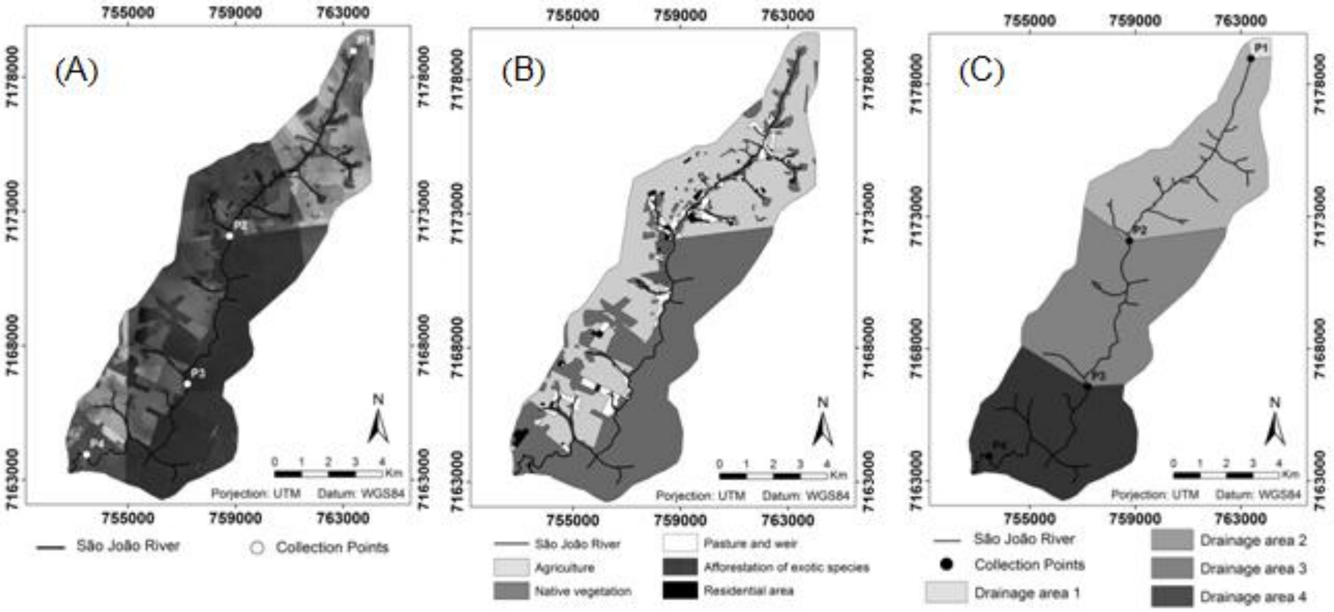

Figure 2. (A) Microbasin of the São João River; (B) Land use and occupation; (C) Drainage areas and sampling sites of the São João River Microbasin.

agriculture (87.57%), and to a lesser extent by native forest (12.42%) found within a radius of approximately 50 m around the source of the São João River.

For sampling site P2, the most prevalent class of land use was agriculture (76.28%); native forest represented just 14.76% for this drainage area, followed by pasture and reservoirs, and residential areas.

Although the percentage of native vegetation around sampling siteP1 was low, it is within the limits established by Article 2 of the Brazilian Forestry Code (Law nº4.771/65) (BRASIL, 1965). The main watercourse feeding sampling site P2 also complied with the minimum limits of the same legislation for plant cover. However, the 11 springs that drain to sampling site P2 are bereft of vegetation on their margins, and display high levels of human activities in their surroundings (Figure 2b); both are factors which invariably influence water quality of the tributaries and consequently the main riverbed.

Stands of exotic tree species (*Eucalyptus globulus* Labill.) are found only within the area draining to sampling site P3, though the major class of land use for this area is native forest (62.69%), with a contribution of 31.87% from agriculture. Four streams run through the drainage area of sampling site P3 to the main riverbed of the São João River, two of these rise and flow through the interior of the Iguaçu National Park until their point of discharge. In contrast, the margins of the third and fourth streams are not protected by a riparian zone, and suffer the effects of heterogeneity of land use and occupation (Figures 2b and c).

Similarly for sampling site P4, two streams that lack riparian zones drain to the main riverbed the alterations caused by agriculture (22.30%), pasture (5.37%), and residential areas (1.56%), in contrast to the 70.76% of native forest, which influences the water quality of the third stream (Figures 2B and C).

Table 2. Summary of the physical, chemical, and biological water quality parameters (mean ± standard deviation) for which limits are specified by legislation (CONAMA 357/05) for samples collected from the sampling sites of the São João and Iguaçu Rivers, from July 2011 to June 2012.

Parameter	P1	P2	P3	L.C.1	L.C.2
TDS (mg/L)	27.75 ± 15.63	36.51 ± 22.8	35.70 ± 23.57	< 500	< 500
TUR (UNT)	12.89 ± 10.08	9.98 ± 4.52	10.92 ± 3.71	< 100	< 40
DO (mg/L)	8.72 ± 1.31	10.54 ± 5.06	10.77 ± 3.76	> 5	> 6
NH_4 (mg/L)	0.41 ± 0.73	0.55 ± 0.68	0.34 ± 0.30	< 10	< 10
NO_2^- (mg/L)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	< 1	< 1
Pt (mg/L)	0.09 ± 0.12	0.41 ± 0.44*	0.16 ± 0.21*	< 0.1	< 0.1
Nt (mg/L)	0.46 ± 0.73	0.58 ± 0.68	0.35 ± 0.31	< 3.7	< 3.7
pH (U. pH)	6.54 ± 1.03	6.87 ± 0.67	7.10 ± 0.59	>6	>6
BOD (mg/L)	1.33 ± 1.09	1.19 ± 0.79	3.07 ± 3.02	< 5	< 3
Cte** (NMP/100ml)	0.27 ± 1.21 ^{a}	2.15 ± 3.89 ^{c}	1.61 ± 3.77 ^{bc}	< 3.4	< 3.4
Cto** (NMP/100ml)	1.06 ± 2.3 ^{a}	2.93 ± 4.68 ^{bc}	2.85 ± 3.98 ^{c}	-	-
Parâmetros	P4	P5	P6	L.C.1	L.C.2
TDS (mg/L)	36.00 ± 29.16	35.91 ± 26.72	37.36 ± 24.76	< 500	< 500
TUR (UNT)	13.62 ± 3.40	9.54 ± 4.54	10.87 ± 5.73	< 100	< 40
DO (mg/L)	11.38 ± 4.84	9.95 ± 3.34	10.96 ± 3.61	> 5	> 6
NO_3^- (mg/L)	0.33 ± 0.27	0.38 ± 0.34	0.36 ± 0.37	< 10	< 10
NO_2^- (mg/L)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	< 1	< 1
Pt (mg/L)	0.16 ± 0.71*	0.20 ± 0.26*	0.26 ± 0.23*	< 0.1	< 0.1
Nt (mg/L)	0.35 ± 0.27	0.40 ± 0.35	0.41 ± 0.38	< 3.7	< 3.7
pH (U. pH)	6.91 ± 0.93	7.04 ± 0.85	7.07 ± 0.73	>6	>6
BOD (mg/L)	0.85 ± 0.62	2.56 ± 2.29	1.49 ± 2.28	< 5	< 3
Cte** (NMP/100ml)	0.35 ± 1.45 ^{a}	0.65 ± 1.82 ^{ab}	0.76 ± 3.09 ^{ab}	< 3.4	< 3.4
Cto** (NMP/100ml)	2.00 ± 4.43 ^{abc}	1.54 ± 2.41 ^{abc}	1.38 ± 3.78 ^{ab}	-	-

Total dissolved solids (TDS), turbidity (TUR), dissolved oxygen (DO), nitrate (NO_3^-), nitrite (NO_2^-), total phosphorus (TP), total nitrogen (TN), pH, biochemical oxygen demand (BOD), thermotolerant coliforms (FC), total coliforms (TC), sampling site (S), CONAMA Legislation 357/05 for rivers of classes I and II (L.C.1), - limit not established under Brazilian law. Means in a line of the table which are followed by the same letter do not differ significantly from one another (Tukey test, $p < 0.05$), * mean value outside the CONAMA 357/05 limits for rivers of class I/II, ** data transformed to $\log_{10}(x+1)$.

Water quality parameters and seasonal variations: Annual means for the physical and chemical parameters for which limits are specified by legislation CONAMA 357/05 (BRASIL, 2008) for rivers of water quality classes I and II are presented in Table 2 for each of the sampling sites. This table also contains the values of thermotolerant coliforms (FC) and total coliforms (TC) and the Tukey test results ($p < 0.05$).

The means for the physical and chemical parameters of the water were compared against the standards established by CONAMA Resolution 357/05 (BRASIL, 2008) and the limits for freshwater of class I (São João River) and class II (Iguaçu River). For sampling sites P2 to P6 the only parameter above the limit specified by the legislation was total phosphorus (upper limit 0.1 mg/L); parameters were within the limits specified (Table 2).

This could be expected, for the São João and Iguaçu Rivers flow through farming and urban areas, respectively, and it is well known that the use of land for agriculture or as pasture within river basins promotes a gradual increase in the concentration of phosphorus in the water (Dodds and Oakes, 2006). Furthermore, the input of allochthonous phosphate-containing materials in these areas is evidence of human actions directly interfering with water quality (Andrade et al., 2007).

Despite the inversion of the proportions of land use assigned to the native forest and agriculture classes between P1 and P4 (Table 1), the significance test applied to the water quality bioindicator FC led to the inference of similarity between the sampling sites (Table 2). This is probably due to mitigation of farming-related impacts by the area of native forest that surrounds the spring feeding P1 (Reserva Particular de Patrimônio Natural de Santa Maria), which is sufficient for the preservation of the stream according to the range determined by current Brazilian legislation (BRASIL, 1965).

From P2 to P3, the drainage area showed an improvement in the preservation of the natural vegetation, with the area given over to farming decreasing by 44.41% and an increase of 47.93% in the area covered by native forest (Table 1). However, sampling sites P2 and P3

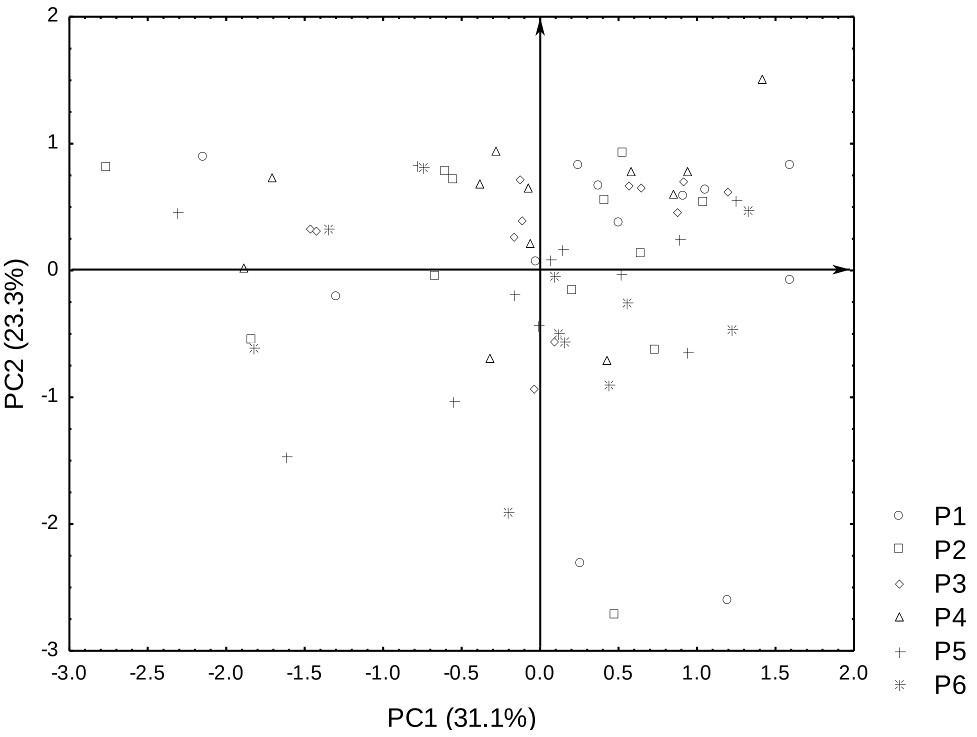

Figure 3. Principal Components Analysis between the axes PC1 and PC2, categorized by the sampling sites of the São João and Iguaçu Rivers, in relation to the physical and chemical parameters of the water (P1 to P6 represent the sampling sites).

showed the highest annual mean values for FC (Table 2). Among the most common sources of microbial contamination (TC and FC) are livestock breeding, areas of pasture, confinement, and application of compost for agricultural purposes, for which evidence was found in the drainage areas of P2 and P3 (Figure 2B) (Gazzaz et al., 2012). Despite the closeness of these sampling sites to common sources of pollution and the lack of a significant difference in the values for thermotolerant coliforms (FC), there was a reduction in the volume of thermotolerant coliforms from 2.15 to 1.61 MPN/100 ml, for the P2 and P3 respectively. This could also indicate the positive influence of recovery of the local vegetation in the drainage areas close to these sampling sites.

Sampling sites P5 and P6 did not show a significant difference in their thermotolerant coliforms concentrations, further there was significant similarity with the most preserved sampling sites (P1 and P4) (Table 2). These results indicate that the continuous flow of the river remains this parameter stable, even with the intense human activity from visits to the Iguaçu Falls, which attracted approximately 1,4 million visitors between the sampling sites in question (P5 and P6) (ICMBio, 2013).

The correlation analyses (Pearson and Spearman) revealed a lack of linear association between the physical, chemical, and biological parameters and the daily, weekly, and monthly rainfall. Further, the multivariate analysis (PCA) did not reveal temporal clustering (seasonality) of the physical and chemical parameters.

The assessment of the associations between the environmental parameters provided evidence for the individual participation of the physical and chemical variables on the quality of the water. The first two axes obtained from the principal components analysis (PCA) were able to explain 54.5% of the environmental variation related to physical and chemical variables of the water from sampling sites (Figure 3).

The variables which negatively influenced axis 1 (PC1) were nitrate, nitrite, and orthophosphate. The second axis (PC2) selected the variables that contributed negatively to its formation, which were ammonia and the ammonium ion.

The groupings formed by sampling sites P1 and P3 (related to axis PC1), and P3 and P4 (related to PC2) may be labelled as the ‘group of dissolved solids’ (Figure 4). These free nutrients play an important role in the ecological processes of the trophic chain of aquatic ecosystems and its distribution in the water column (Guedes et al., 2012). The sampling sites (P1, P3 and P4) most influenced by the group of dissolved solids are those with the greatest integrity of riparian forest, which through being in partial or total contact with the reserve were consequently less influenced by different forms of land use.

The occurrence in the water column of nitrogen-containing compounds that influenced the axes in this

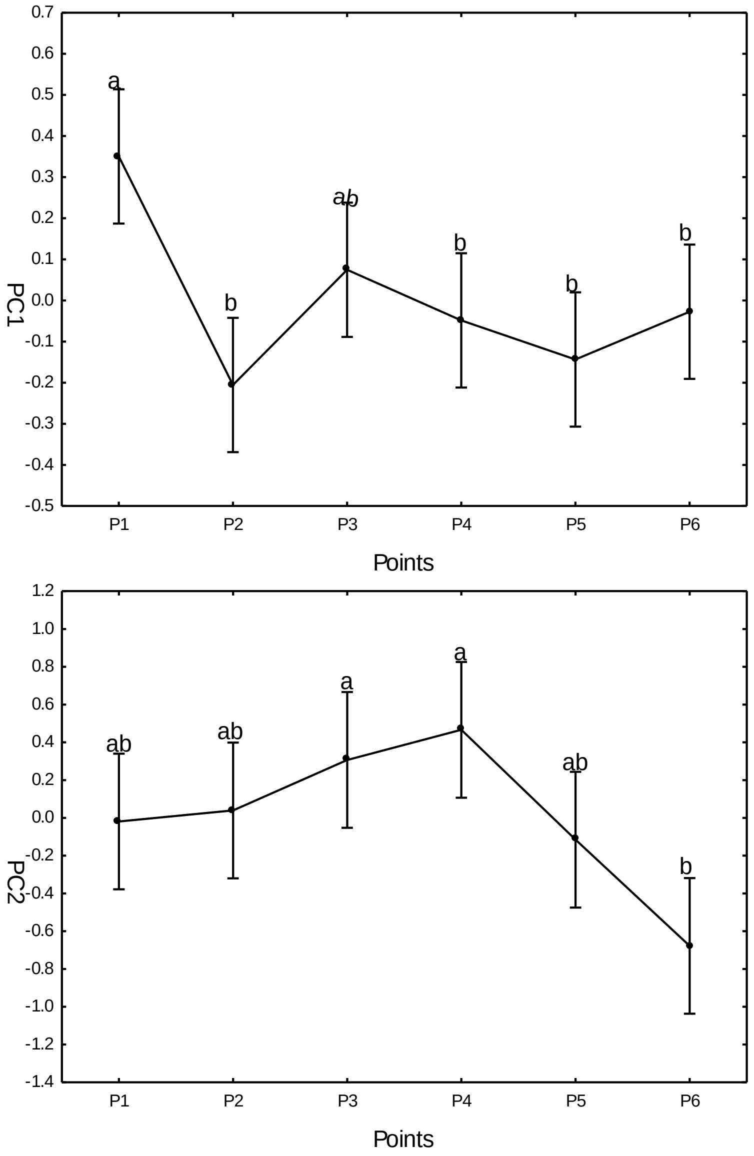

Figure 4. Two-way ANOVA between the axes from the principal components analysis (PCA) and the sampling sites of the São João and Iguaçu Rivers (means followed by the same letter do not differ significantly from one another: Tukey test, $p < 0.05$).

research may be directly linked to the processes of production and decomposition of organic matter (Esteves and Amado, 2011). It is evident that the input of phosphate-containing nutrients found on the axes could vary according to the different climatic conditions and vegetation of the drainage basin, for they are mainly generated through the deposition of particulate material, living organisms, and organic matter derived from leaf decomposition within the riparian zone (Esteves and Panosso, 2011).

Furthermore, the main source of energy and nutrients in continental aquatic ecosystems is basically formed from leaf detritus coming from the riparian zone. These allochthonous inputs are fundamental to the understanding of the energy flow and for the maintenance of the metabolism of lotic aquatic ecosystems, especially in Neotropical regions (Esteves and Junior, 2011).

The cluster grouping analysis revealed that the sampling sites could be divided into two large groups: ‘G1’, corresponding to the São João River and ‘G2’ corresponding to Iguaçu River. Within these groupings, three distinct groups (A, B and C) were formed, between which there was greater heterogeneity (Figure 5). The first grouping (B) was composed of P2 and P3, both highly affected by human activities. Sampling site P1 and P4 (C), close to the boundary of the Iguaçu National

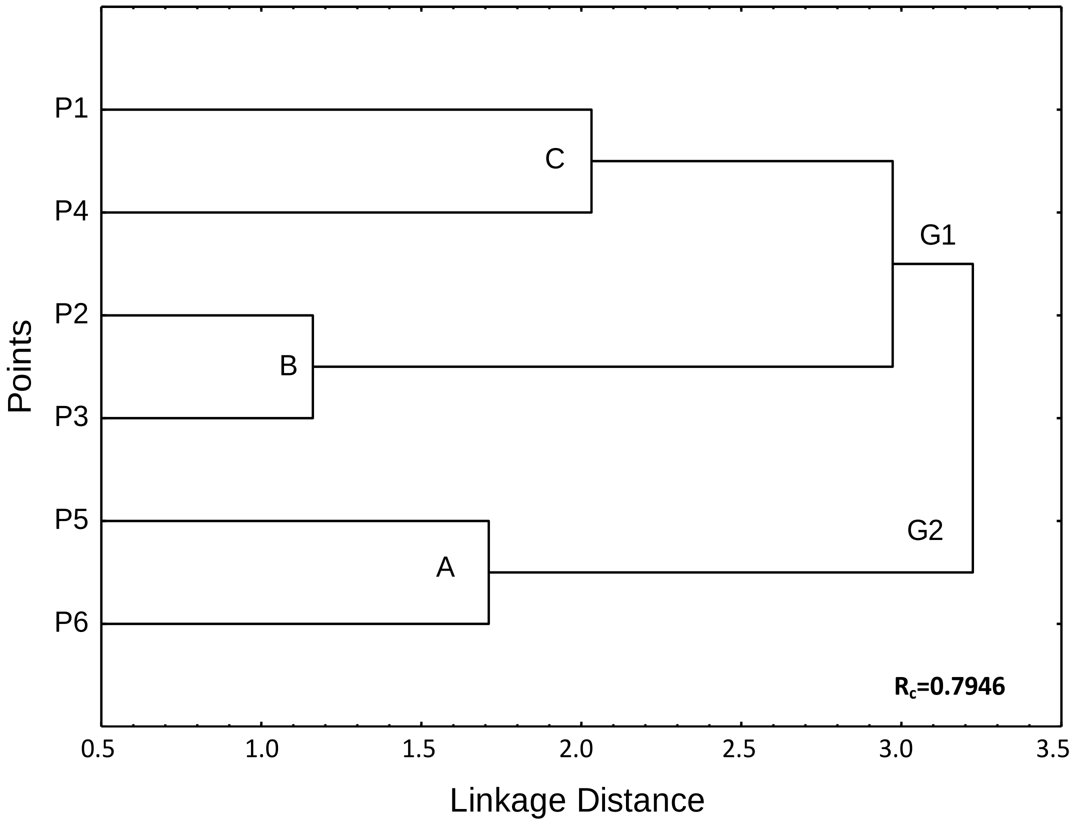

Figure 5. Dendrogram from the Cluster grouping analysis of the physical and chemical parameters for the sampling sites of the São João and Iguaçu Rivers. Rc: Cophenetic correlation coefficient.

Park, and appeared separately from the others, probably as a result of the self-purification processes occurring along the length of the São João River and action protection of riparian vegetation formed by the RPPNSM (P1) and ParNaIguaçu (P4).

Sampling site P1 and P4 (grouping C) stood out from the others, which constitute the São João River region in contact with the protected forest, and when compared to the other sampling sites, it displayed unique physical, chemical, and biological characteristics. The large group 2 was composed of sampling sites P5 and P6 (grouping A), both these sites are located on the Iguaçu River within the Iguaçu National Park, and displayed similar environmental characteristics.

Based upon the similarity evidenced through the cluster analysis confirmed by Analysis Not Parametric similarity to $p < 0.05$ for P1-P4 points, P2-P3 and P5 and P6 and cophenetic correlation coefficient (Rc = 0.7946), it was possible to establish that the analyses of water from sampling sites P1, P2 and P6 would have been sufficient for the purposes of assessing the water quality from these locations. Thus, it will be possible to optimize the sampling in future monitoring campaigns, reducing the cost and labour without decreasing the reliability of the data.

Conclusions

The São João River Microbasin is composed of five different classes of land use: Native forest, pasture and reservoirs, agriculture, residential areas, and areas of afforestation by exotic species. Although native forest and agriculture were the predominant land use classes in this microbasin, this was not found to be true for the drainage areas of the sampling sites. The physical, chemical, and biological variables of water quality did not show correlations with rainfall patterns (seasonality). However, there were relations between the quality of the water resources and the location of the sampling sites. The values of the physical and chemical variables of the assessed sampling sites were within the limits required by the current Brazilian legislation, when they were not influenced by water coming from streams bereft of riparian vegetation. The influence of these streams can also be seen through a direct proportionality, with the significant increase in the biological indicators. It was established that the integrity of the aquatic ecosystems suffered no significant interference form the different types of land use, provided that the riparian zone remained well preserved. However, evidence was found for local alterations in the integrity of the aquatic ecosystems and in the quality of the water for the sampling sites fed by streams lacking the protection from riparian forests.

Conflict of Interest

The authors have not declared any conflict of interest.

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